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THE CHEMICAL COMPOSITION OF FEEDING STUFFS AVAILABLE IN CANADA[1]

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Aside from a few early papers (Gamble and Harcourt (1), Shutt and Hamilton (7, 8), Shutt (6)), there appears to be no published data giving a survey of the composition of Canadian feeding stuffs. Therefore, it was thought that a compilation of this kind would be of considerable value to those interested directly or indirectly in the feeding of livestock.

Presented in this paper are the results of a survey carried out during the period 1939 to 1943 by the Department of Animal Nutrition, whereby 373 samples, representing 80 types of feeding stuffs were analysed for organic and mineral nutrients. The samples were from feeds which were available in Canada and which, with only a few exceptions, were produced in this country. The descriptive names of these feeding stuffs (meat meal, meat scrap, etc.) used herein are those under which the products were marketed.

METHODS OF ANALYSIS

The analyses for ash, crude protein ($N \times 6.25$), ether extract, and crude fibre were conducted according to the methods of the A.O.A.C. (4). Official methods were also used for moisture, with the exception of milk products which were dried to constant weight at 100° C. under atmospheric pressure.

The percentage of nitrogen-free-extract was obtained for each individual sample by subtracting from 100 the percentage of moisture, crude protein, ether extract, crude fibre and ash. It should be pointed out that where the analyses on certain samples in a group of feeding stuffs were incomplete the sum of the average values may only approximate 100%.

Destruction of organic matter, preparatory to analysing for calcium and phosphorus, was accomplished by digestion of the samples with nitric and perchloric acids as described by Gerritz (2). Calcium was determined by precipitation as calcium oxalate and titration with potassium permanganate. Phosphorus was determined by the method of King (3), the intensity of the blue colour being measured with a Cenco-Sheard-Sandford photelometer.

For the determination of manganese, Schaible's modification (5) of the Willard-Greathouse method (9) was employed. The values are expressed as parts per million.

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In the case of four types of feed where only one sample of each was available and determinations were not made for some of the constituents, the data were completed by the use of average values compiled by Morrison.[3]

DISCUSSION

In Table 1 the average, minimum and maximum values obtained for the various constituents are given for each type of feeding stuff. Where complete analysis was not obtained for every sample in a group, the number of samples analyzed is placed in parentheses just above the average value. It was thought that the calculation of the standard deviations was not warranted since in most groups the number of samples available for analysis was relatively small. However, the maximum and minimum values do provide a reasonably good indication of the wide variation in composition which, in many cases, was encountered within a group of feeding stuffs of similar type.

These variations may be attributed only in part to differences in the moisture content since they were not reduced appreciably by a comparison of the data on a "moisture-free" basis.[4] It is likely that one or more of such factors as climatic and soil conditions, time of harvesting and method of curing, variety of grains and forage crops and milling and extraction processes were responsible for most of these variations.

Since average values are widely used for the purpose of estimating the nutrient content of individual feeding stuffs of mixed rations, it is important to emphasize that the composition of individual lots of the same type of feed may differ widely. Obviously, the use of averages may, in certain instances, lead to markedly erroneous results.

A large number of feeding stuffs come under the Feeding Stuffs Act and hence carry a maximum or minimum guarantee with respect to their content of certain nutrients. The use of such feeding stuffs for the preparation of a mixed ration of a specific composition is thus greatly simplified. However, the value as guaranteed may not always provide a reliable measure of the true content of the nutrient in question. Two examples may be cited from the present survey, one involving crude protein and the other, crude fibre content. Sixteen samples of meat meal, guaranteed to contain not less than 55% protein were analysed and six of these samples were found to be appreciably below this guarantee. Three of these six samples were below guarantee even when the protein was calculated on a "moisture-free" basis. Among 34 samples of various wheat by-products, nine samples exceeded the maximum limits for crude fibre for their class as designated under the Feeding Stuffs Act of 1937.

In view of the above considerations, it would seem advisable that, where a close approximation of the composition of a feed is desired, a chemical analysis should be carried out and average values should not be relied upon. On the other hand, where facilities are not readily available for such an analysis, average data, as presented in Table 1, can be of considerable worth, provided they are used with due regard for the number of samples analysed and the range in values which was found.

[3] F. B. Morrison, Feeds and Feeding, Appendix, 20th Edition, 1936. Reproduced by permission of the publishers.

[4] "Moisture-free" data are not presented in this paper.

SUMMARY

Eighty types of commercial feeding stuffs available in Canada were analysed for moisture, crude protein, ether extract, crude fibre, ash, calcium, phosphorus and manganese.

The significance and practical value of this compilation has been discussed.

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TABLE 1.—THE CHEMICAL COMPOSITION OF FEEDING STUFFS AVAILABLE IN CANADA

Feeding stuff*	No. of samples	Moisture	Crude protein	Ether extract	Crude fibre	N.F.E.	Ash	Calcium	Phosphorus	Manganese
		%	%	%	%	%	%	%	%	p.p.m.
Animal Products										
Blood Meal	5	Av. 10.0	84.4	1.1	0.7	1.1	2.8	0.33	0.30	5
		Min. 5.8	82.0	0.2	0.4	0.3	1.8	0.19	0.21	2
		Max. 11.4	85.8	4.1	1.0	2.0	3.9	0.54	0.41	12
Bone Meal (Feeding)	7	Av. 6.7	25.8	4.7	—	—	59.7	24.99	10.86	2
		Min. 3.6	22.1	2.1	—	—	54.1	21.40	10.21	1
		Max. 9.1	28.3	12.8	—	—	63.5	37.40	11.50	3
Bone Meal (Steamed)	5	Av. 3.3	7.6	1.6	—	—	80.5	29.10	14.26	8
		Min. 2.8	5.7	0.4	—	—	73.6	25.50	13.20	4
		Max. 4.2	12.2	4.0	—	—	84.5	31.60	15.00	16
Buttermilk Powder				(8)†	(8)	(8)	(9)			(10)
(Dried)	12	Av. 13.7	32.8	3.9	0.2	40.8	7.5	1.31	0.93	3
		Min. 4.2	30.0	1.3	0.1	31.9	5.7	0.97	0.90	0
		Max. 22.5	38.0	6.2	0.5	55.5	9.8	1.85	1.05	7
Casein				(14)	(3)	(3)	(17)			
	18	Av. 9.0	79.8	0.4	0.1	4.5	3.3	0.78	1.43	3
		Min. 7.2	68.8	0.1	0.05	0	1.7	0.05	0.75	0
		Max. 12.0	84.5	1.7	0.2	7.5	7.6	1.33	1.55	4
Cod Liver Meal	3	Av. 9.3	48.4	28.5	0.3	10.7	2.7	0.13	0.76	6
		Min. 6.0	43.7	18.3	0.1	6.8	2.1	0.03	0.68	5
		Max. 14.2	55.4	34.3	0.5	15.2	3.2	0.27	0.85	7
Cracklings (Cow)	1	10.9	57.2	9.4	1.2	0	23.0	7.42	3.77	28
Cracklings (Curb process)	1	6.7	53.5	8.8	2.8	0.6	27.6	9.00	6.46	14
Cracklings (Expeller process)	1	5.7	60.5	10.2	2.3	0.9	20.3	6.35	3.35	8
Cracklings (Horse)	1	6.0	57.7	9.7	0.9	3.3	22.4	7.16	3.78	3
Fish Meals	7	Av. 8.6	62.4	6.4	0.6	4.1	17.9	5.33	3.28	12
(Unspecified)		Min. 6.4	56.1	1.4	0.4	0.0	10.2	2.65	2.18	6
		Max. 10.6	70.3	15.2	1.1	8.7	24.7	8.05	4.48	18
Fish Meal (White)	5	Av. 8.4	67.2	1.9	0.5	1.5	20.7	6.76	3.69	11
		Min. 5.4	58.3	1.6	0.2	0.0	15.0	5.83	2.24	7
		Max. 10.4	71.6	2.3	0.8	3.1	26.8	8.55	4.73	20
Fish Meal (Herring)	1	7.6	68.3	4.2	0.3	9.9	9.6	2.49	2.08	10
Fish Meal (Pilchard)	3	Av. 9.6	65.8	9.1	0.8	2.5	13.6	4.04	2.72	9
		Min. 9.4	62.1	5.6	0.3	0.0	11.1	3.05	2.29	9
		Max. 9.9	71.1	11.9	1.5	5.3	15.1	4.58	3.04	9
Liver Meal	5	Av. 6.9	65.0	19.0	1.3	2.6	5.2	0.64	1.26	8
		Min. 6.2	61.0	18.1	1.0	0.0	4.4	0.07	1.09	6
		Max. 7.2	67.8	19.9	1.6	8.0	6.9	1.52	1.57	10
Lung and Liver Meal	1	Av. 6.8	61.1	21.9	1.7	0.7	7.8	1.68	1.47	6
Meat Meal (55% protein or over)	16			(9)	(9)	(9)	(12)			
		Av. 6.4	55.1	10.3	2.4	1.6	23.1	8.25	3.48	7
		Min. 4.1	49.5	9.2	0.8	0.0	17.5	7.72	1.01	2
		Max. 8.1	61.4	11.9	3.2	7.2	27.3	11.99	6.00	29

* The descriptive names of the feeding stuffs are those under which the products were marketed.
† Number of samples analysed.

TABLE 1.—THE CHEMICAL COMPOSITION OF FEEDING STUFFS AVAILABLE IN CANADA —*Continued*

Feeding stuff	No. of samples	Moisture	Crude protein	Ether extract	Crude fibre	N.F.E.	Ash	Calcium	Phosphorus	Manganese
		%	%	%	%	%	%	%	%	p.p.m.
Meat Meal (below 55% protein)				(15)	(15)	(9)	(16)			(16)
	17	Av. 6.7	49.3	11.7	3.7	2.4	27.3	9.04	4.79	7
		Min. 5.2	45.3	9.4	1.7	0.0	21.3	7.07	3.10	3
		Max. 8.5	54.6	14.9	5.1	5.8	34.2	11.21	5.59	13
Meat and Bone Meal	1	5.7	46.0	12.4	2.1	1.5	32.3	11.1	5.40	5
Meat Scrap	5	Av. 6.5	51.8	11.9	2.0	0.9	26.9	9.43	4.18	11
		Min. 4.5	45.3	11.1	1.6	0.3	17.9	5.61	2.15	6
		Max. 9.5	58.8	13.1	2.6	2.1	35.4	13.30	6.58	27
Milk Albumin (dried)	3	Av. 8.0	52.0	0.9	1.0	12.8	25.4	8.19	4.37	32
		Min. 7.5	51.8	0.7	0.7	12.1	25.3	7.71	4.35	31
		Max. 8.4	52.4	1.0	1.2	13.4	25.5	8.48	4.40	33
Skimmilk Powder				(4)	(4)	(4)				(5)
	6	Av. 9.4	32.9	0.4	0.1	50.3	5.9	1.13	0.96	1
		Min. 5.8	30.4	0.2	0.1	47.8	4.5	1.07	0.88	0
		Max. 12.7	35.8	0.6	0.1	54.7	6.9	1.26	1.08	1
Tankage										(10)
	11	Av. 8.1	56.6	8.5	2.3	3.4	23.1	7.44	3.90	8
		Min. 6.7	45.6	6.0	1.1	0.0	17.4	5.51	1.99	5
		Max. 11.7	64.1	14.2	5.6	4.3	31.4	10.40	5.31	11
Whey Powder	1	11.3	24.2	3.5	0.2	54.6	6.3	1.26	0.94	3
Cereals and Their By-products										
Barley				(6)	(6)	(6)	(6)			
	7	Av. 12.1	12.4	2.2	5.6	66.0	2.2	0.08	0.46	16
		Min. 11.8	11.7	1.8	4.5	65.2	1.8	0.05	0.31	14
		Max. 13.1	13.6	2.7	7.7	67.0	2.5	0.19	0.50	18
Barley Feed				(1)	(1)	(1)				
	2	Av. 10.3	15.5	4.7	10.6	54.7	4.8	0.09	0.45	28
		Min. 9.9	15.1				4.2	0.07	0.43	25
		Max. 10.8	15.9				5.4	0.10	0.46	31
Buckwheat	2	Av. 11.5	11.4	2.8	11.0	61.4	1.9	0.08	0.34	31
		Min. 11.3	11.3	2.6	10.8	61.3	1.9	0.07	0.33	30
		Max. 11.7	11.6	2.9	11.1	61.5	2.0	0.08	0.35	32
Corn (African)				(2)	(2)	(2)				
	3	Av. 10.8	10.5	4.1	1.7	71.6	1.2	0.01	0.26	7
		Min. 10.1	10.1	3.6	1.5	69.4	1.0	0.01	0.23	7
		Max. 11.7	11.1	4.7	1.8	73.8	1.4	0.02	0.30	8
Corn (American yellow)				(4)	(4)	(4)				
	5	Av. 12.0	10.1	4.0	2.0	70.6	1.3	0.01	0.27	8
		Min. 10.3	8.2	3.8	1.6	66.8	1.0	0.01	0.24	5
		Max. 13.1	13.4	4.3	2.2	72.7	1.6	0.02	0.28	14
Corn (Argentine)				(1)	(1)	(1)				
	2	Av. 11.3	10.0	4.8	1.6	70.6	1.3	0.02	0.30	8
		Min. 10.7	9.7				1.3	0.02	0.30	7
		Max. 12.0	10.3				1.4	0.02	0.31	8

TABLE 1.—THE CHEMICAL COMPOSITION OF FEEDING STUFFS AVAILABLE IN CANADA
—Continued

Feeding stuff	No. of samples	Moisture	Crude protein	Ether extract	Crude fibre	N.F.E.	Ash	Calcium	Phosphorus	Manganese
		%	%	%	%	%	%	%	%	p.p.m.
Corn (Canadian yellow)				(7)	(7)	(7)				
	10	Av. 11.8	9.3	4.1	1.8	71.6	1.3	0.01	0.29	6
		Min. 9.5	8.3	2.0	1.5	68.9	1.1	0.00	0.26	4
		Max. 13.7	11.8	5.2	2.2	74.0	1.3	0.02	0.36	11
Corn (White)				(2)	(2)	(2)				
	3	Av. 12.7	10.1	4.1	1.9	68.8	1.1	0.01	0.25	5
		Min. 10.6	8.6	3.7	1.8	66.3	0.9	0.01	0.20	3
		Max. 14.4	12.5	4.5	2.0	71.4	1.2	0.01	0.29	6
Corn Germ Meal	1	9.8	19.6	5.8	10.4	52.3	2.1	0.04	0.47	19
Corn Gluten Feed				(4)	(4)	(4)				
	6	Av. 10.4	26.0	3.8	7.6	49.0	4.6	0.10	0.74	17
		Min. 8.2	25.3	3.5	7.1	46.2	3.0	0.06	0.54	9
		Max. 14.7	27.0	4.3	8.3	51.1	7.9	0.15	0.99	32
Corn Meal (Table)	1	13.9	7.6	1.3	0.3	76.5	0.5	0.00	0.11	2
Hominy Feed (White)	6	Av. 10.0	10.4	8.2	4.2	64.1	2.1	0.01	0.52	13
		Min. 9.1	9.6	7.6	3.8	54.7	1.8	0.01	0.40	10
		Max. 10.9	11.1	8.7	5.2	70.7	2.5	0.03	0.58	24
Hominy Feed (Yellow)	3	Av. 10.7	10.0	7.7	3.9	65.8	1.9	0.02	0.50	11
		Min. 10.0	8.8	6.6	3.8	64.4	1.6	0.01	0.43	10
		Max. 11.5	10.8	8.2	4.1	68.3	2.2	0.03	0.57	13
Oats (whole)				(7)	(7)	(7)				
	8	Av. 10.5	12.3	3.9	10.4	60.0	2.7	0.10	0.32	32
		Min. 7.3	11.5	2.7	6.2	57.7	2.4	0.05	0.28	20
		Max. 12.5	13.6	4.9	12.5	66.8	2.9	0.13	0.42	46
Oats (Rolled)	1	9.5	16.1	7.3	1.5	64.0	1.7	0.05	0.38	33
Oat Dust	1	8.1	18.0				4.7	0.14	0.49	95
Oat Groats	7	Av. 10.2	17.9	5.9	2.5	60.3	1.9	0.07	0.43	30
		Min. 8.7	14.8	3.6	1.9	57.5	1.7	0.04	0.37	20
		Max. 11.2	21.6	7.5	3.0	64.5	2.1	0.10	0.46	35
Oat Hulls	1	5.53	7.05	1.2‡	30.6‡	51.2‡	5.7	0.12	0.18	20
Oat Meal	1	9.7	16.2	6.5	1.5	64.3	1.9	0.05	0.41	31
Oat Meal (Pinhead)	1	11.3	18.5	7.1	2.0	59.4	1.6	0.09	0.45	29
Oat Middlings				(4)	(4)	(4)				
	5	Av. 8.8	14.8	6.4	3.6	64.4	2.2	0.08	0.53	45
		Min. 7.2	10.2	4.9	2.1	62.2	1.5	0.05	0.37	23
		Max. 10.7	17.2	7.9	5.3	68.1	3.5	0.14	0.78	90
Rice Bran	1	12.8	8.2	13.4	10.5	57.7	7.3	0.45	1.19	176
Rye Middlings	1	11.2	17.5	2.7	5.4	60.3	2.9	0.06	0.63	44
Wheat				(11)	(11)	(11)	(12)			
	13	Av. 11.8	13.3	1.8	2.6	68.9	1.5	0.06	0.39	33
		Min. 8.6	9.8	1.0	2.2	65.7	1.0	0.03	0.29	12
		Max. 13.6	15.5	2.4	3.1	72.4	2.2	0.17	0.41	48

TABLE 1.—THE CHEMICAL COMPOSITION OF FEEDING STUFFS AVAILABLE IN CANADA —*Continued*

Feeding stuff	No. of samples	Moisture	Crude protein	Ether extract	Crude fibre	N.F.E.	Ash	Calcium	Phosphorus	Manganese
		%	%	%	%	%	%	%	%	p.p.m.
Wheat Bran				(7)	(7)	(7)				
	9	Av. 10.6	16.3	4.5	11.6	52.7	5.8	0.11	1.27	113
		Min. 8.8	14.1	3.1	10.0	48.8	5.1	0.10	1.15	78
		Max. 12.2	20.4	5.4	13.8	58.1	6.4	0.16	1.47	140
Wheat Feed Flour	1	11.7	16.7	2.2	1.5	66.5	1.5	0.03	0.38	26
Wheat Germ				(5)	(5)	(5)				
	6	Av. 10.0	28.6	12.7	2.4	42.5	4.1	0.06	1.00	135
		Min. 7.3	25.3	8.4	2.0	27.9	3.2	0.04	0.76	91
		Max. 11.7	31.3	24.5	3.4	49.5	4.7	0.11	1.14	194
Wheat Germ (Defatted)	1	6.7	38.6	2.7	2.1	44.4	5.6	0.05	1.25	176
Wheat Germ Middlings				(2)	(2)	(2)				
	3	Av. 11.2	27.8	10.6	3.3	43.2	4.0	0.07	0.98	126
		Min. 9.1	25.0	9.3	2.7	40.7	3.7	0.03	0.91	117
		Max. 12.4	29.8	11.9	3.8	45.6	4.5	0.11	1.11	141
Wheat Middlings				(6)	(6)	(6)				
	7	Av. 10.8	19.2	4.9	4.8	57.9	3.0	0.07	0.69	86
		Min. 9.5	16.6	3.8	2.3	55.7	2.2	0.04	0.46	52
		Max. 11.8	21.9	5.4	6.1	63.3	3.7	0.09	0.80	108
Wheat Shorts				(6)	(6)	(6)				
	7	Av. 9.5	17.2	4.9	7.8	56.4	4.1	0.09	0.87	106
		Min. 5.9	15.2	4.0	5.9	51.8	3.5	0.08	0.47	79
		Max. 11.7	19.2	5.5	9.5	58.7	5.0	0.10	1.19	145
Forages										
Alfalfa (Dehydrated)				(23)	(23)	(22)	(23)	(34)	(34)	(34)
	35	Av. 9.7	17.4	2.8	24.3	39.8	7.1	1.71	0.21	34
		Min. 5.8	11.4	1.6	15.8	35.4	4.9	1.01	0.13	12
		Max. 13.0	26.5	4.5	30.0	43.3	11.1	2.31	0.54	55
Alfalfa (Sun cured)				(4)	(4)	(4)				
	5	Av. 10.9	15.6	2.0	26.6	38.7	6.0	1.55	0.17	33
		Min. 8.8	11.8	1.7	23.4	36.7	5.0	1.15	0.16	27
		Max. 13.8	18.2	2.5	29.6	41.8	6.9	1.83	0.18	39
Cereal Grass (Dehydrated)				(12)	(12)	(12)	(13)			
	15	Av. 8.8	20.4	5.6	16.6	35.8	12.4	0.72	0.61	29
		Min. 6.7	18.5	3.5	12.6	29.8	6.0	0.36	0.22	5
		Max. 10.9	27.5	11.5	19.3	40.7	16.4	1.58	1.71	61
Miscellaneous Feeding Stuffs										
Alfalfa Silage (Dried)	1	5.8	17.2	2.4	29.4	27.4	7.9	1.57	0.86	—
Beet Pulp (Dried)	2	Av. 11.3	9.2	0.6	20.0	56.4	2.5	0.69	0.09	25
		Min. 11.0	9.1	0.5	19.4	55.8	2.0	0.66	0.08	18
		Max. 11.6	9.3	0.8	20.6	56.9	3.0	0.72	0.10	33
Brewers' Grains (dried)	1	8.0	22.8	4.0	24.9	37.5	2.9	0.19	0.33	33

TABLE 1.—THE CHEMICAL COMPOSITION OF FEEDING STUFFS AVAILABLE IN CANADA‡
—Concluded

Feeding stuff	No. of samples	Moisture	Crude protein	Ether extract	Crude fibre	N.F.E.	Ash	Calcium	Phosphorus	Manganese
		%	%	%	%	%	%	%	%	p.p.m.
Copra Meal	1	9.9	21.1	6.5	18.8	38.7	5.0	0.11	0.58	61
Cottonseed Meal	6	Av. 8.2	40.1	6.4	10.5	28.2	6.4	0.16	1.35	18
		Min. 7.0	37.6	5.7	9.5	26.8	6.1	0.13	1.29	17
		Max. 9.5	42.1	6.9	11.6	30.1	6.9	0.20	1.39	20
Distillers' Dried Corn	1	7.9	28.8	4.0	13.8	43.5	1.8	0.03	0.43	14
Distillers' Dried Mixed Grains	1	7.4	30.7	6.0	13.5	41.0	1.4	0.04	0.28	35
Distillers' Dried Wheat	1	8.1	24.1	—	—	—	—	0.05	0.55	15
Feed Screenings	1	12.1	13.8	2.6	4.9	64.7	2.0	0.08	0.35	28
Flaxseed	1	8.2	20.8	36.4‡	5.9‡	24.2‡	3.6‡	0.34	0.60	15
Kelp Meal	1	13.4	8.2	1.4	11.6	50.7	—	1.14	0.16	2
Linseed oil meal				(7)	(7)	(7)	(7)			
	9	Av. 9.9	36.5	6.7	8.0	33.6	4.7	0.29	0.90	41
		Min. 8.5	33.2	5.4	6.7	30.6	4.0	0.10	0.67	20
		Max. 11.1	41.0	8.3	10.0	36.8	5.1	0.33	1.58	49
Malt Sprouts	2	Av. 11.0	26.7	1.4	13.7	41.9	5.4	0.25	0.76	35
		Min. 8.3	23.7	1.4	13.3	41.7	5.2	0.18	0.74	15
		Max. 13.8	29.7	1.4	14.1	42.0	5.5	0.32	0.78	55
Peas	2	Av. 12.6	24.1	1.1	4.7	55.4	2.1	0.09	0.43	30
		Min. 12.1	23.0	1.1	4.4	54.8	1.7	0.08	0.27	13
		Max. 13.1	25.2	1.1	5.0	56.1	2.5	0.10	0.59	47
Rapeseed Meal	1	7.6	32.7	5.1	11.7	30.4	6.3	0.47	1.00	42
Soybean Germ Oil Meal	1	6.9	38.5	—	—	—	3.7	0.12	0.64	24
Soybean Oil Meal				(9)	(9)	(9)	(10)			
	13	Av. 9.8	43.8	3.0	5.3	32.4	5.0	0.24	0.59	28
		Min. 8.1	40.5	0.3	0.9	29.8	4.3	0.14	0.58	24
		Max. 11.3	48.7	5.1	6.4	36.1	5.8	0.31	0.80	34
Starch	1	7.9	0.4	5.5	—	—	0.0	0.01	0.02	0
Sunflower Seeds	2	Av. 7.8	15.4	25.0	29.6	19.8	2.4	0.17	0.49	9
		Min. 7.2	14.3	22.6	28.7	17.4	2.0	0.15	0.47	9
		Max. 8.5	16.5	27.4	30.5	22.2	2.8	0.18	0.52	10
Sunflower Seed Meal	1	7.8	40.1	18.3‡	10.9‡	21.8‡	5.5	0.24	1.22	5
Tares	1	11.9	25.8	1.0	4.7	54.1	2.5	0.15	0.36	29
Yeast				(3)	(3)	(3)	(3)			
	7	Av. 7.3	49.8	0.8	2.7	37.0	7.6	0.09	1.64	6
		Min. 4.4	38.8	0.5	2.1	29.1	7.4	0.05	1.21	5
		Max. 11.1	57.5	1.3	3.6	43.0	7.9	0.13	2.19	10

‡ Values reproduced from Feeds and Feeding by Morrison, 1936, with the permission of the publishers.

COMMON SCAB OF POTATO IN DRY AND WET SOILS[1]

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The relation of the water content of the soil to the incidence of common scab of potato is of general interest because of its direct effect on the vegetative growth and hyphal fragmentation of *Actinomyces scabies* (Thaxter) Gussow and also its effect on the associated soil bacteria and fungi.

It is not difficult to compare the growth of this pathogen in dry, medium, and wet soils, since inoculum of the fungus in a sterilized black loam soil produces an excellent vegetative growth under aseptic conditions.

EXPERIMENTAL

The soil containers for demonstrating growth of *A. scabies* in soils of different moisture content may be tight-fitting Petri plates, test tubes, or, if photographs are desired, small metal boxes, each fitted with a removable glass window and a hole in the cover. Metal boxes 5 × 5 × 7 inches were used for obtaining the data listed in Table 1. Soils of required moist-

TABLE 1.—THE OBSERVED VEGETATIVE GROWTH OF *Actinomyces scabies* IN A STEAM STERILIZED BLACK LOAM SOIL AT DIFFERENT SOIL MOISTURES

Soil water content*	Comparative growth rating†					Condition of soil
	Days after seeding soil					
	2	3	5	6	9	
%	%	%	%	%	%	
19	0	0	70	80	80	Very dry
22	0	0	80	90	90	
26	0	0	90	90	90	
30	0	0	100	100	100	Optimum
35	0	0	100	100	100	
39	0	0	20	60	90	Wet

* Approximate percentage of moisture holding capacity.
† Average of duplicates.

ure content were made up according to plan and steam sterilized in Erlenmeyer flasks, then aseptically poured into the sterilized boxes. When the container was about one-half full, a narrow band of soil next to the window was seeded with a very small quantity of soil-spore mixture, which was obtained by shaking a small amount of soil over a well matured culture of *A. scabies*. The containers were then filled to the top, the hole plugged with cotton, and the edges of the glass sealed with paraffin wax. The incubation temperature was 23° C. The moisture content of the soils in the different series was approximately 19, 22, 26, 30, 35, and 39% of their water-holding capacity. The first soil of this series was too dry for plant

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growth and the last one too wet to handle easily. Comparative ratings of the vegetative growth of *A. scabies* were made on the fifth, sixth, and ninth days after seeding with spores—a value of 10 representing the best growth observed on each date.

Five days after seeding, the observed growth in the dry soil was 30% poorer than in the soil of optimum or over-optimum moisture content, but about 70% better than that in the wet soil. However, one day later growth in the dry soil was only 25% better, and after three days the situation was reversed, with growth actually 12% better in the wet soil (Figure 1). Evidently the pathogen will grow surprisingly well in a soil too dry for plant growth, although it must be remembered that in a closed container the soil air would tend to be more humid than under field conditions. However, according to Lebedeff (3) the soil atmosphere is usually saturated under field conditions, except the surface layer, which occasionally becomes air-dry. Undoubtedly *A. scabies* grows faster and produces aerial hyphae more profusely at optimum moisture content, although hyphal fragmentation is favoured by dry conditions and by aging.

FIGURE 1. Above—Excellent growth of *Actinomyces scabies* in wet soil (39% m.h.c.). Below—Unseeded soil in which there are a few colonies from seeded strip.

DISCUSSION

The effect of soil moisture content on the incidence of potato scab may be conveniently reviewed under two headings, viz.: (*a*) growth of the pathogen in the absence of biological antagonism; and (*b*) in its presence.

The data in Table 1 are in general agreement with the results reported (7) earlier from growing *A. scabies* in a sterilized soil of different moistures and counting the colonies obtained from soil dilutions. Lutman, Livingstone and Schmidt (4) obtained the greatest number of colonies of *A. scabies* during winter from a field soil of high moisture content. Goss (2) concluded from his studies carried out in the greenhouse that "counts of *Actinomyces* in soil dilution plates and on soil slides did not check with each other, nor did either show any consistent relation to soil moisture or to scab". Sanford (6), 1923, reported severe scab in a naturally infested dry field soil an almost clean tubers in the same soil maintained fairly wet, and as a result of other laboratory experiments stated that "favourable conditions for growth of abundant inoculum and for subsequent growth would not be found either in an excessively dry or in a very moist soil, but in one of intermediate moisture content". It was thought "that in a dry soil sufficient moisture transpired from the tuber to enable spores adhering closely to the surface or about the lenticels to germinate and cause infection". In view

of the amount of growth noted in a dry soil in Table 1 the foregoing explanation still seems to have much merit. Of course if the soil were very dry before tuber formation, conditions would be definitely inimical to the viability and persistence of the pathogen and may result in a clean crop. Soil aeration in a dry soil under normal conditions would be excellent, and it is obvious that the oxygen supply would still be adequate for the pathogen until the soil became quite wet.

Let us now briefly consider antibiosis to potato scab in dry and wet soils. This factor was suggested (7) in 1926. Various degrees of compatibility and antagonism between *A. scabies* and a number of common soil bacteria were observed on solid and in liquid media, and it was concluded that these relationships would also occur in the soil. Subsequently (9) some common soil-inhabiting bacteria were shown to suppress, others kill, and still others to have no appreciable ill effect on the pathogen in a sterilized soil medium. Millard and Taylor (5) believed that *A. praecox* suppressed (by competition) the growth of *A. scabies* in their pot experiments, where grass cuttings were mixed in the soil, and they thought that bacteria might exercise a similar effect. Goss (2) reported no control from *A. praecox* in a heavily infested, steam sterilized soil, and Sanford (8) also obtained no control in a steam sterilized soil with or without green rye added. Dippenaar (1) found more *Actinomyces* than bacteria on slides buried in a dry soil, and the opposite in wet soil—which is not surprising. He concluded that the operation of bacterial antagonism to *A. scabies* in a wet soil explained why he obtained more scab in a dry soil than in one of high moisture content. Goss (2) obtained reduction of scab in a sterilized soil by adding organic manure or filtrates of unsterilized soil, and concluded that the reduction was due to a resulting increase of soil saprophytes.

Evidently biological antagonism must be accepted as an important factor in the production of inoculum of *A. scabies* in field soils. The evidence is also strong that it may be even more effective in reducing or preventing infection of the host. Obviously in a dry soil this antagonism would be severely reduced, whereas it would tend to become increasingly effective as moisture conditions and available nutrients became more and more suitable for the growth of soil saprophytes. This is especially true if soil bacteria are, in general, more antagonistic than fungi to the pathogen, as now seems probable. However, there seems no apparent reason why severe scab may not occur in soils of higher moisture content if the development of antagonistic micro-organisms in sufficient amount does not happen to be favoured by the existing conditions.

SUMMARY

The vegetative growth of *A. scabies* in steam sterilized black loam of six moisture contents, ranging from dry to wet, was observed during 9 days after 'seeding' the soil. It was always best at about optimum soil moisture, but surprisingly good in both dry and wet soils. At the beginning (5 days), growth in the wet soil lagged far behind that in the dry soil, but 4 days

later it was equally good or better. Thus, in the absence of effective antagonism from associated saprophytes, severe scab may be expected in soils high in moisture content, as well as in drier soils.

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FUSARIUM SAMBUCINUM FKL. F. 6 WR. AS A PATHOGEN OF SOME SPECIES OF THE *CUCURBITACEAE*[1]

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At Brooks, Alberta, in August, 1942, a number of vegetable marrow plants were severely wilted. Examination of the crown region of these plants revealed an extensive buff-coloured dry rot. A species of *Fusarium* was isolated from the stems and found to be very pathogenic to marrow. Dr. W. L. Gordon, of the Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba, kindly identified the isolate as *Fusarium sambucinum* Fkl. f. 6 Wr. Only one reference (3) to this form as a pathogen of cucurbits was noted in the literature, and in that case it was reported as a cause of pumpkin fruit rot. The results of various tests of the pathogenicity of *F. sambucinum* f. 6 to marrows, squash, pumpkin, muskmellon and cucumbers are reported in the present paper.

METHODS AND RESULTS

Experiment I

In this experiment the effect of seed treatment upon infection by *F. sambucinum* f. 6 in various soils was investigated. Five samples of local soil were taken, viz., A, B and C, black loam in fallow, after wheat, and under sod, respectively; sample D, a leached soil, was taken from under poplar trees; and E, one rich in leaf mould, from under willow shrubs.

Each of the above mentioned soils was placed in 6-inch flower pots in the greenhouse, and 75 grams of soil-grown inoculum of the pathogen was mixed with the top 300 grams of soil. Fifty seeds of Imperial Long Green cucumber, treated with 1% by weight of 2% Ceresan dust, were planted in each of four replicated pots of each soil sample. A like number of inoculated pots were planted to 50 untreated seeds.

The average percentage emergence of the treated and untreated seed, respectively, 10 days after planting, was as follows: Soil A—16.4, 5.4; Soil B—8, 8.4; Soil C—17.4, 20.4; Soil D—38, 6.4; and Soil E—18, 18. The germination of this seed in a moist chamber was 94%.

The pathogen was very destructive in all cases and the effect of seed treatment was not so marked as it might have been under a less severe test. Seed treatments in Soils A and D appeared to be much more effective in reducing infection than was the case in the other three soil samples. The soils highest in organic matter (C and E) seemed to exert a protective effect against the pathogen independent of seed treatment. Moreover, the seedlings were most vigorous in Soil C and least vigorous in Soil B.

Experiment II

This experiment was an extension of the study on the effect of seed treatment. Twenty seeds of Imperial Long Green cucumber, 8 of Sugar pumpkin, 5 of Green Hubbard squash, and 5 of White Trailing marrow

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were planted in 6-inch pots in the greenhouse. Forty grams of soil-grown inoculum of *F. sambucinum* f. 6 was mixed with 300 grams of soil from the top of each pot, and used to cover the seed. The seed for four replicated pots was treated with 1% by weight of 2% Ceresan by shaking in a flask and separating the seed from the excess dust. The seed for four additional replicated pots was untreated. All pots were placed in large metal trays to which water was added occasionally in an attempt to ensure equal moisture in all pots. In Table 1 the data on living plants are shown for 11 and 15 days, respectively, after planting.

TABLE 1.—EFFECT OF SEED TREATMENT ON THE INFECTION OF CUCURBIT SEEDLINGS BY *Fusarium sambucinum* F. 6 AT PERIODS OF 11 AND 15 DAYS AFTER PLANTING

Host plant	Living plants as percentage of seeds planted			
	Treated seed		Untreated seed	
	11 days	15 days	11 days	15 days
	%	%	%	%
Cucumber	35	40	12.5	17.5
Pumpkin	97	9	69	0
Squash	65	70	60	30
Marrow	100	10	0	0

An examination of columns 1 and 3 indicates a marked beneficial effect from seed treatment at the 11-day stage in the case of cucumber, pumpkin and marrow. The beneficial effect of seed treatment on squash is noted at the end of the fifteenth day. Marrow and pumpkin appear to be the most susceptible and squash the least, also it is apparent that the fungus is extremely pathogenic. In a greenhouse test of this nature, conditions are made to favour the pathogen at the expense of the host plant. It is probable that under field conditions, in a naturally infested soil, seed treatment would protect cucurbit seedlings much more effectively than was the case in pots.

Experiment III

In this experiment a comparison was made of the pathogenicity of three cultures of *F. sambucinum* f. 6 isolated from an alfalfa root, a potato tuber, and a diseased marrow stem. Twenty seeds of cucumber, 20 of muskmelon, 5 of marrow, and 10 of pumpkin were planted in pots. Forty grams of soil-grown inoculum of the three fungus isolates was mixed with 300 grams from the surface soil of four replicated pots in each case. A like number of control pots received no inoculum. The pots were placed in trays to which water was added when necessary. The number of living plants at the end of 15 days is indicated in Table 2.

Of the three strains of *F. sambucinum* f. 6 under test, only the strain from marrow demonstrated pathogenicity, which was very marked. The criterion of pathogenicity (2) was used to identify physiologic races of *F. niveum* on watermelon. Apparently a similar situation exists in the

TABLE 2.—RELATIVE PATHOGENICITY OF THREE STRAINS OF *Fusarium sambucinum* F. 6 TO CUCUMBER, MUSKMELON, MARROW AND PUMPKIN

Host plant	Living plants 15 days after planting as percentage of seeds planted			
	Strains isolated from			Control
	Alfalfa	Potato	Marrow	
	%	%	%	%
Cucumber	77	75	29	75
Muskmelon	78	80	0	75
Marrow	85	85	0	85
Pumpkin	72	72	0	70

case of *F. sambucinum* f. 6. Miller (1) has recently reported on the wide variation in pathogenicity to muskmelons of mutants of a *Fusarium* culture similar to *F. bulbigenum* var. *niveum* f.2.

Experiment IV

The effect of soil temperature on the pathogenicity of *F. sambucinum* f. 6 to cucumber and pumpkin was noted in this experiment. One-gallon crocks of soil were suspended in tanks of water at constant temperatures of 25°, 20°, 15°, and 11° C. Four of the crocks at each temperature were inoculated by mixing 200 grams of soil-grown inoculum of *F. sambucinum* f. 6 with 800 grams of the top soil from the crock. Twenty seeds of cucumber were planted 1 inch deep in this mixture. The soil in four control crocks was not infested. The crocks were covered with discs of waxed paper until emergence of the seedlings. After 20 days' growth the plants were removed and 12 pumpkin seeds planted in the same soil without the addition of any more inoculum. The effect of temperature on host and pathogen is indicated in Table 3.

TABLE 3.—EFFECT OF SOIL TEMPERATURE (CENTIGRADE) ON THE PATHOGENICITY OF *Fusarium sambucinum* F. 6 TO CUCUMBER AND PUMPKIN SEEDLINGS

Days after planting	Living plants as percentage of seed planted							
	Non-inoculated soil				Inoculated soil			
	25°	20°	15°	11°	25°	20°	15°	11°
	%	%	%	%	%	%	%	%
	Cucumbers							
7	85	18	X	X	20	0	X	X
10	85	50	X	X	38	19	X	X
12	86	51	4	X	36	21	X	X
16	86	53	9	X	14	13	3	X
20	86	55	10	X	8	10	4	X
	Pumpkins							
5	87	17	X	X	85	17	X	X
9	87	85	85	X	93	95	88	X
13	87	85	87	X	0	15	88	X

X — No emergence.

Soil temperatures from 11° to 15° C. were too low for satisfactory growth of cucumber seedlings. The pathogen was increasingly virulent from 15° to 25° C. Most of the seedlings in the infested soil were killed before they could emerge.

It is indicated that pumpkin is more tolerant than cucumber to low soil temperatures. Since a good emergence of the former occurred by the ninth day at 15° C., a comparison of the survival data of the pumpkin and cucumber plants in the infested soil indicates that the lack of fresh inoculum at the time of planting the pumpkin seed definitely reduced early mortality. However, the sudden wilting of the seedlings between the ninth and thirteenth days shows that the pathogen was still active in the replanted soil.

Experiment V

The data obtained from the greenhouse work were supplemented by field experiments. Seed of pumpkin, squash, and marrow were planted May 19 in 12-foot rows, replicated four times. Twenty-five seeds per row were planted and 150 grams of soil-grown inoculum of *F. sambucinum* f. 6 was added with the seed in the inoculated series. The weather subsequent to seeding was unsuitably cool and wet for these plants. By July 5 there were 76, 34, and 43 living plants of pumpkin, squash, and marrow, respectively, in the control rows, whereas the corresponding figures for the inoculated rows were 2, 8, and 0. Only 1 pumpkin and 3 squash plants were living by September 1. When the inoculum was placed in contact with the seed only a few plants escaped destruction in the seedling stage, and most of these succumbed at intervals up to and including the fruiting stage. In each case the plant wilted suddenly. Disease symptoms in the field and greenhouse were similar. That is to say, the tissue of the stem below the soil surface becomes necrotic, dry, breaks off easily, and is of a cream-buff colour.

The growth of the mycelium of the pathogen through the soil was demonstrated. Cucumber seeds were planted without inoculum added. When the plants were past the seedling stage (July 23), 50 grams of soil-grown inoculum of *F. sambucinum* f. 6 was placed at depths of 2 to 4.5 inches from the stem. At September 28, none of the 25 plants under test had wilted down, but all showed varying degrees of browning of the foliage, and few produced fruit. The pathogen was recovered in every case from the crown tissue by plating sections in nutrient agar.

SUMMARY

Fusarium sambucinum f. 6 was isolated from the stems of wilted marrow plants, and, in experiments in greenhouse and field, shown to severely attack and destroy the lower stem-portion of marrow, squash, pumpkin, muskmelon, and cucumber plants. Other isolates of this fungus from diseased potato tubers and alfalfa roots were not pathogenic to any of the cucurbits mentioned. Apparently this is the first record of a physiologic form of this species being a major pathogen to cucurbits. The gross symptoms of the disease are indicated.

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RIBOFLAVIN CONTENT OF CANADIAN FEEDSTUFFS[1]

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The importance of riboflavin in livestock rations, particularly for poultry and swine, has been established and widely emphasized within recent years. As a consequence, this vitamin is one of the factors which must be taken into consideration in the compounding of these rations. It is well-known that certain ration components such as milk products and some high-potency fermentation products or by-products will supply the greater proportion of the ration requirement of riboflavin. Recently, pure crystalline riboflavin has been used successfully to replace a large part of the riboflavin from these sources. It is also true, however, that many other commonly used feedstuffs do contain appreciable amounts of riboflavin, and, depending upon the proportions in which they are included in rations, these materials can make small to moderate contributions toward meeting the required riboflavin levels in the rations. The amounts of the high-potency riboflavin sources required to be added will, therefore, depend to some extent upon the basic constitution of the rations. Another aspect which must be borne in mind is the variability in riboflavin content of feedstuffs of the same type.

The published literature records the results of many analyses of feedstuffs for their riboflavin content. As has been pointed out elsewhere (1), many published tables of vitamin values of feedstuffs quote only "average" values without any indication of the wide variations which may be encountered from sample to sample. Information of any kind on the riboflavin values of Canadian feedstuffs has been very limited.

This report presents information on the riboflavin content of miscellaneous Canadian feedstuffs with the major exception of milk products. The data reported herein have been accumulated during a period of approximately four years as a part of an analytical survey of Canadian feedstuffs. The results of the riboflavin studies on milk products have already been published (1). The results of the general chemical analyses of miscellaneous feedstuffs are reported in another paper (2).

EXPERIMENTAL

Many of the samples herein discussed were obtained directly from producers and distributors in response to requests for samples for this study. A much larger number represented bulk lots of feedstuffs obtained by departments of the College for use in routine and investigational feeding. The remaining few samples came to hand in connection with other aspects of the normal activities of the Department of Animal Nutrition. In all cases the samples represented feeding-stuffs produced or offered for sale in Canada. Complete information on the source and approximate date of origin for a majority of the samples is on file.

The microbiological assay method employed for the assays has been described previously (1). The remarks made at that time concerning the

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relative reliability of the results are equally applicable to the present work. All determinations were conducted on the materials as received, and the results are expressed on that basis without any consideration of water content.

RESULTS

The results of the assays are consolidated in Table 1. Space does not permit the reporting of the potencies of the individual samples, but the inclusion in the table of the low and high observed values in addition to the mean values does serve to illustrate the extent of the variations encountered. Standard deviations have been calculated in those cases where 5 or more samples of a particular type of feedstuff were assayed. It should be pointed out that the values for the standard deviations are intended to serve only as indications of the variations in riboflavin potency of the samples studied, thereby compensating somewhat for the omission of the individual figures.

All the entries in the table were made on the basis of the identifying descriptions received with the samples. This means that the result for each material is included under the classification in which it was sold or offered for sale, regardless of whether or not the sample met the standards for that classification. Some few of the samples represented mixtures of unspecified proportions of two or more products. While these mixtures may not be typical of any well-defined classes of feedstuffs, they were included as a matter of interest. It should be pointed out also that the value of the figures reported for the "unspecified" samples of fish meal, liver meal and alfalfa meal is limited somewhat by the absence of complete information on the origin of these samples.

Perhaps the dominant feature of these results is the moderate to extreme variations in the riboflavin content of the "fair" to "good" riboflavin sources such as meat meal, fish meal, liver meal, alfalfa meal, cereal grass and yeast. The obvious variability of these feedstuffs emphasizes again the possible dangers in the use of so-called "average" riboflavin figures in the construction of rations.

It is possible that the variability in riboflavin content of processed feedstuffs might be reduced by more careful control of processing and by improvements in the methods involved. The establishment and enforcement of a system of guaranteed riboflavin levels for the important riboflavin carriers would undoubtedly be of value to the consumer, in that such would eliminate to some extent the uncertainty at present involved in the use of these materials.

SUMMARY

A total of 241 samples, representing a wide variety of types of feedstuffs of vegetable and animal origin (excluding milk products), was assayed for riboflavin content by the microbiological method. Fish meal, liver meal, meat meal, alfalfa meal, cereal grass, soybean oilmeal, and yeast were the feedstuff types which were represented by the largest numbers of samples. Low, high and mean values were recorded in all cases.

Rather wide variations in riboflavin content were found in many of the classes of materials.

Feedstuff	No. of samples	Riboflavin content (micrograms/gram)			
		Low	High	Mean	Standard deviation
Feeds of Animal Origin:					
Blood meal	8	0.1	1.1	0.5	0.30
Bone meal (feeding bone)	2	0.2	0.4	0.3	—
Cracklings:					
Cow	1	—	—	4.4	—
Horse	1	—	—	3.5	—
Fish meal:					
Pilchard	1	—	—	3.8	—
Whitefish	2	5.1	7.8	6.5	—
Unspecified	13	3.7	9.6	6.1	1.96
Hoof and horn meal	1	—	—	0	—
Liver meal:					
Cod	3	17	28	21	—
Pork	1	—	—	31	—
Unspecified	12	29	64	49	11.8
Lung meal	1	—	—	11	—
Lung and liver meal	3	13	21	18	—
Meat meal	44	2.4	8.2	4.6	1.42
Tankage	6	1.4	3.1	2.2	0.81
Feeds of Vegetable Origin:					
Alfalfa meal:					
Dehydrated	21	6.4	20	14	3.5
Suncured	5	7.0	11	9.3	1.69
Unspecified	4	5.3	11	8.7	—
Barley:					
Whole grain	3	1.3	1.3	1.3	—
Beet pulp	1	—	—	0.7	—
Cereal grass	13	11	18	15	2.5
Cereal grass + dehydrated alfalfa	1	—	—	15	—
Cereal + legume grass	2	12	15	13.5	—
Corn:					
Corn distillers' dried grains	1	—	—	3.1	—
Corn germ oil meal	1	—	—	3.0	—
Corn gluten feed	2	1.5	1.9	1.7	—
Hominy	3	1.3	2.8	2.0	—
Hominy feed	1	—	—	1.8	—
Whole grain	7	1.0	1.5	1.1	0.37
Cottonseed oil meal	3	2.3	3.8	3.1	—
Linseed oil meal	6	1.8	2.9	2.1	0.53
Oats:					
Groats	2	1.1	1.1	1.1	—
Whole grain	2	1.1	1.3	1.2	—
Rapeseed oil meal	1	—	—	1.8	—
Soybean oil meal	19	2.3	3.8	2.7	0.52
Sunflower seed oil meal	1	—	—	3.0	—
Wheat:					
Bran	6	2.6	3.6	3.0	0.40
Germ	2	5.3	5.7	5.5	—
Shorts	7	2.8	3.8	3.0	0.75
Wheat distillers' dried grains	2	2.1	3.3	2.7	—
Wheat distillers' dark grains	2	3.4	3.9	3.7	—
Whole grain	2	1.0	1.2	1.1	—
Yeast	22	2.7	89	36	20.6

ACKNOWLEDGMENT

The authors wish to express their appreciation of the co-operation shown by the many producers, distributors and others who furnished samples for this survey. The technical assistance of Miss E. D. Sparke in the conducting of many of the assays is gratefully acknowledged.

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PHENOTHIAZINE IN CODLING MOTH CONTROL[1]

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Experiments with phenothiazine as a summer spray for control of the codling moth (*Carpocapsa pomonella* L.) were begun during 1937 and carried on until 1944 in the Okanagan Valley of British Columbia. As this project has been discontinued for the present, a review of the work may be useful.

LITERATURE

First reference to the high insecticidal value of phenothiazine was that of Campbell, Sullivan, Smith and Haller (1). As prepared in the laboratory by one of these authors, thiodiphenylamine (phenothiazine) appeared to be even more toxic to mosquito larvae than rotenone. The following year Smith and Siegler (6) experimented with phenothiazine for control of codling moth and reported that it was fully as effective as lead arsenate. Further laboratory work by Siegler, Munger and Smith (5) demonstrated that the initial toxicity of pure phenothiazine to codling moth was superior to that of lead arsenate. They concluded that as its cost should be relatively low for an organic compound, it should hold promise as an orchard insecticide. Newcomer (4), after two seasons' experience with phenothiazine in the field, stated that it was more effective in controlling the codling moth than any other material tried at Yakima, Washington. Used with soap it resulted in half as many wormy apples and one-tenth as many stings as an equal amount of lead arsenate with soap. He reported, however, that it reduced the red colour of apples to some degree and in hot weather had a tendency to irritate the skin of orchard workers.

Phenothiazine is not wetted by water and herein lies one of the difficulties in using the compound as an orchard insecticide. It is readily wetted by oil but it was thought for some time that a phenothiazine-oil mixture could not be used as a summer application because of the danger of foliage injury. Accordingly, adjuvants such as soap or sodium lauryl sulphate were suggested, but experiments showed that adhesion of phenothiazine was unsatisfactory with these substances. Not until it was used with stove oil did its exceptional toxicity to codling moth larvae become fully evident. It was discovered at the same time, that phenothiazine-stove oil mixture caused no more injury than phenothiazine alone.

According to Zukel (7), the action of phenothiazine on the cockroach is entirely one of contact. On the other hand, A. D. Heriot[3], of the Vernon Laboratory, found that undiluted phenothiazine applied as a contact dust to codling moth pupae had no effect, whereas a dust of 5% sodium arsenite produced 98% mortality.

EXPERIMENTS IN BRITISH COLUMBIA

The field work with phenothiazine for codling moth control in British Columbia has been done in the Okanagan Valley where irrigation is a

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[2] Entomologist in charge.

[3] Unpublished work.

necessity and the temperature occasionally exceeds 100° F. The codling moth has a heavy second generation and in some years a partial third. At the present time its control in most orchards necessitates three lead arsenate and two cryolite spray applications, and in some cases fixed nicotine-oil is also used for late sprays. Casein-lime is the adjuvant with both lead arsenate and cryolite in order to ensure a powdery deposit that will not be difficult to remove with fruit wipers. Heavy spraying is the rule, in many orchards about one gallon spray liquid being applied in each treatment for each box of maximum crop production.

Hoy (3) has reviewed the experimental use of phenothiazine for codling moth control in British Columbia up to 1942. Results reported by Hoy, together with those for 1943 and 1944, are brought together in Table 1. The experimental plots varied in size from 6 to 10 trees; variety as a rule was McIntosh. Check plots adjoined the experimentally treated plots.

Data with respect to materials are:

Stove oil: 31.7 S.S.U. Vis. 100° F., 86% U.R. (A.O.A.C.)
Summer oil: 69 S.S.U. Vis. 100° F., 77% U.R. (A.O.A.C.)
Casein-lime: casein 10%, hydrated lime 90%.

Phenothiazine was obtained each year from Du Pont Chemicals, through Canadian Industries Ltd.

In 1937 and 1938 the phenothiazine was not micronized and contained a wetting agent of unknown composition. Control of codling moth was somewhat similar to that from lead arsenate-casein-lime on a pound-for-pound basis. A micronized product was obtained in 1941 and when wetted with stove oil and dispersed with soap (monoethanolamine oleate) this very finely divided material appeared to be about twice as effective as the phenothiazine applied previously. In 1942 when it was used in the same way but at reduced concentration and in all applications instead of the last two or three, it gave even more effective control.

Soap for dispersion of oil-wetted phenothiazine was not entirely satisfactory. It produced an unstable mixture that adhered strongly to the spray tank and spray hoses so that the amount of insecticide deposited on the fruit was less than indicated by its concentration in the tank. A hydrophilic colloid, casein-lime, was substituted for soap in 1943. That year, phenothiazine 1 pound per 100 Imperial gallons, wetted by stove oil 1 quart, when used in all cover sprays, resulted in better codling moth control than the standard growers' schedule consisting of lead arsenate 4 pounds, casein-lime 4 ounces, in early sprays and cryolite 4 pounds, casein-lime 4 ounces, in later sprays.

Micronized phenothiazine wetted with stove oil or summer oil and dispersed with casein-lime was applied to seven separate plots involving about 1.5 acres of large trees in 1944. Once again it proved at least four times as effective as lead arsenate or cryolite.

Although in all the British Columbia work the number of stung fruits was, with one exception, less in the phenothiazine plots than in the lead arsenate or cryolite checks, it did not drop to a degree comparable with the 1 : 10 ratio for total stings noted by Newcomer (4).

TABLE 1.—PHENOTHIAZINE IN CODLING MOTH CONTROL

Year	Materials per 100 Imp. gal.	Cover sprays applied	Percentage injured fruits	
			Stung	Wormy
1937	Phenothiazine 2 lb. (unknown wetting agent) Lead arsenate 3.2 lb., casein-lime 4 oz.	4 4	13.4 15.6	21.0 5.1
1937	Phenothiazine 3 lb. (unknown wetting agent) Lead arsenate 3.2 lb., casein-lime 4 oz.	4 4	2.5 1.5	7.5 2.5
1938	Phenothiazine 3.75 lb. (unknown wetting agent) Lead arsenate 3.75 lb., casein-lime 4 oz.	Last 2 of 5 5	3.0 6.3	2.2 2.4
1941	Phenothiazine (micronized) 1.8 lb., stove oil 1 qt., monoethanolamine oleate 0.5 lb. Lead arsenate 3.75 lb., casein-lime 4 oz. Cryolite 3.75 lb., casein-lime 4 oz.	Last 3 of 6 4 2	5.6 9.8	9.8 10.9
1942	Phenothiazine (micronized) 1.8 lb., stove oil 1 qt., monoethanolamine oleate 0.5 lb. Lead arsenate 3.75 lb., casein-lime 4 oz. Cryolite 3.75 lb., casein-lime 4 oz.	5 3 2	0.9 1.8	1.4 3.5
1943	Phenothiazine (micronized) 1 lb., stove oil 1 qt. casein-lime 4 oz. Lead arsenate 4 lb., casein-lime 4 oz. Cryolite 4 lb., casein-lime 4 oz.	5 3 2	1.0 4.4	7.6 25.7
1944	Phenothiazine (micronized) 1 lb., stove oil 1 qt. casein-lime 3 oz. Cryolite 4 lb., casein-lime 4 oz.	5 5	0.6 0.7	1.8 2.8
1944	Phenothiazine (micronized) 0.5 lb., stove oil 1 pt., casein-lime 3 oz. Cryolite 4 lb., casein-lime 4 oz.	5 5	0.4 1.1	5.0 3.6
1944	Phenothiazine (micronized) 1 lb., stove oil 1 qt., casein-lime 4 oz. Cryolite 4 lb., summer oil 1 qt. Cryolite 4 lb., summer oil 2 qt.	First 4* of 5 3 2	4.6 8.8	8.2† 8.8
1944	Phenothiazine (micronized) 0.5 lb., stove oil 1 pt., casein-lime 4 oz. Cryolite 4 lb., summer oil 1 qt. Cryolite 4 lb., summer oil 2 qt.	First 4* of 5 3 2	4.8 7.4	6.2 9.8
1944	Phenothiazine (micronized) 0.5 lb., summer oil 1 pt., casein-lime 4 oz. Cryolite 4 lb., summer oil 1 qt. Cryolite 4 lb., summer oil 2 qt.	First 4* of 5 3 2	4.6 8.8	7.8 8.8

* Last cover spray fixed nicotine-summer oil.

† Infestation on these trees much heavier in 1943 than on those of succeeding two phenothiazine plots. That may have accounted for there being no apparent improvement in control in 1944 twice with the concentration of phenothiazine.

The most effective phenothiazine mixture was prepared by stirring phenothiazine into stove oil and in turn beating this mixture into an equal quantity of water-casein-lime mixture. The whole was than poured into the spray tank while the tank was being filled and with agitators in operation. In such a spray mixture the phenothiazine-stove oil is at first dispersed in huge and most unpromising flocs, but providing agitation is

adequate, the flocs gradually become smaller. No difficulty was experienced with the mixture either in stationary or portable equipment.

According to Cutright (2), phenothiazine favours development of the European red mite (*Paratetranychus pilosus* C. & F.) in Ohio. During 1944 this effect was also noted in British Columbia. For example, in one plot where phenothiazine was used, the average number of European red mites per leaf on September 5 was 33.6. The adjoining check plot sprayed throughout the season with cryolite-casein-lime (itself a mixture apparently favouring mite development) had an average number of 19.1 mites per leaf. A second phenothiazine plot averaged 41.8 mites and its adjoining check plot, 13.9 mites per leaf. Differences were consistent from tree to tree. In another orchard, it was apparent that phenothiazine also favoured development of the Pacific mite (*Tetranychus pacificus* McG.), although no population records were taken. If phenothiazine should come to be generally used for codling moth control, its unfavourable effect in so far as orchard mites are concerned will have to be taken into account. Evidently that effect may be offset to a large degree by use of fixed nicotine-summer oil for second brood application.

Under Okanagan Valley conditions, it is believed that micronized phenothiazine, if available at a reasonable price, could effectively replace lead arsenate which now is used only in early sprays. Since late applications of phenothiazine have a tendency to affect fruit coloration, second-brood phenothiazine sprays seem less suitable. Incidentally, elimination of lead arsenate from the Okanagan spray schedule is definitely desirable not merely because that compound leaves a highly objectionable residue on the fruit, but because it results in a toxic soil condition if heavily applied year after year.

SUMMARY

1. Experiments with phenothiazine for control of the codling moth (*Carpocapsa pomonella* L.) were carried on in British Columbia from 1937 to 1944. They have been briefly reviewed.

2. The phenothiazine used from 1937 to 1941 had about the same effect as lead arsenate against codling moth. The particle size of this material was evidently too great. When micronized, however, and used with a small quantity of stove oil, phenothiazine pound-for-pound was about four times as effective as lead arsenate.

3. Phenothiazine evidently favoured the development of European red mite (*Paratetranychus pilosus* C. & F.) and Pacific Mite (*Tetranychus pacificus* McG.).

4. Should micronized phenothiazine become available at a reasonable price it might help to eliminate lead arsenate from the British Columbia spray schedule for apples. It seems preferable to use it in early cover sprays rather than in the later applications.

ACKNOWLEDGMENTS

B. Hoy, Provincial Fruit Branch, Kelowna, B.C. collaborated in all work with summer sprays and many of the experimental data are from his plots. Oil specifications were determined by J. M. McArthur, Dominion

Division of Chemistry, Summerland, B.C. Also acknowledged is the work of E. P. Venables, A. D. Heriot and Harry Andison of the staff of the Vernon Laboratory, who did much of the spraying and fruit checking.

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FURTHER BACTERIOLOGICAL STUDIES RELATING TO EGG DRYING[1]

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In a previous publication (9) the standards and procedures employed in the bacteriological control of Canadian dried eggs for export to Britain were described, together with some of the results for 1943. Studies conducted during the latter half of 1943 with the direct microscopic count (10) indicated that this method had considerable value in reflecting the care given the melange before drying. On the other hand, analysis of the results obtained in testing for the presence of coliform organisms and *Escherichia coli* indicated little or no correlation with plate counts of viable organisms, direct microscopic counts, the presence of *Salmonella* species or the results of plant sanitation surveys. The determination of coliform organisms and *E. coli* in Grade A powder was therefore dropped for 1944, a direct microscopic count of 2,000,000 per gram being substituted for it.

RESUME OF RESULTS OF 1944 OPERATIONS

As will be evident from the data in Table 1, the over-all picture as judged by the plate count of viable organisms was very satisfactory. The improvement in average counts over 1943 paralleled improvements brought about in the drying plants following plant sanitation surveys, supplemented by routine checks conducted by the resident inspectors using the Burri slant technique (8). With the direct microscopic counts, on the other hand, a very different picture was obtained. While gratifyingly low during the first four months, they then rose to quite high levels, remaining so until December. Had our analyses been confined to the plate count, we would have remained ignorant of the changed situation.

During the fourth week of May, unusually high direct microscopic counts appeared with dramatic suddenness in the powder from 3 of the 5 Western drying plants (Table 2). In each instance the high count was due to the presence of a short, plump rod, occurring in chains of up to 10 cells, and easily mistaken for a streptococcus. For convenience, this was referred to as the Y organism. It was never isolated from samples of powder received at Ottawa, while attempts to isolate it from numerous samples of melange and powder at the plants soon after the outbreaks met with no success. Plating powder on media containing sterile unheated egg, and incubating plates at temperatures ranging from 38° F. (3.3° C.) to 112° F. (44.4° C.) failed to bring about any significant increase over the standard plate count (1, 9), again suggesting that the organism did not survive the drying process.

In investigating this outbreak, samples were obtained from the start and finish of 3 days' drying at the two plants (A and C) where the Y organism first showed up, to determine whether or not any "build-up" in

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count took place during the day's operations. (Previous experience had indicated the value of this practice in seeking the cause of high counts.) However, the results (Table 3) failed to indicate any consistent increase in count during the day's operations.

Because the appearance of the Y organism coincided with the change to a different batch of North's stain (1, 10), it was thought the explanation might lie in the ability of the new batch of stain to bring out this organism on stained smears, which previous batches had failed to do. However, subsequent comparative tests with old and new stains showed the Y organism to stain equally well with either, so this hypothesis had to be discarded.

The high counts due to the Y organism disappeared as suddenly as they had appeared. The organism appeared in powder made at 4 of the 5 plants in Western Canada, and was never seen in more than two carlots from any one plant. All available evidence suggested that the sporadic appearance of this organism in such large numbers could be attributed to its having invaded the contents of a small percentage of eggs; these being held at higher than average temperatures for longer periods than is customary, the organisms multiplied extensively without causing sufficient change in the appearance or odour of the eggs to enable the breaker to detect and discard them.

That high counts could be due to the eggs themselves, and not to faulty plant practice, was difficult to believe in view of the previous findings on the bacterial content of shell eggs as broken out in 2 Canadian drying plants (6, 7) supplemented by periodical counts at breaking and drying plants thoughout Canada. Such findings had confirmed the conclusion reached by previous investigators that good quality shell eggs contain relatively few bacteria. However, evidence accumulated since May, 1944 has necessitated some modification of this view.

Shortly after the epidemic of high counts in the West, there was a general rise in the level of microscopic counts on powder from all over the country. This is clearly seen in Figure 1, showing the microscopic counts of consecutive carlot samples of Grade A powder. The general level of counts continued to rise until October, following which there was some decline. On the other hand, this seasonal rise was not evident in the plate counts (Figure 2 and Table 1). Since plant sanitation and practices were generally superior to those of the previous year, it seemed most unlikely that these higher levels of microscopic counts could be attributed to faulty plant operations.

The first indication that high counts might be coming from the eggs themselves was obtained in one of the Western plants at the end of May, 1944. This plant (Plant E) had had trouble with high counts for some weeks prior to the appearance of the Y organism (Table 2). Investigation revealed that, because of the extreme shortage of cold storage facilities, eggs had been broken directly out of cars, the temperature of which at times had exceeded 60° F. Melange prepared during the day was pumped into portable 80-gallon holding tanks and run into a cold room held at around the freezing point. Some of this melange would be held for up to

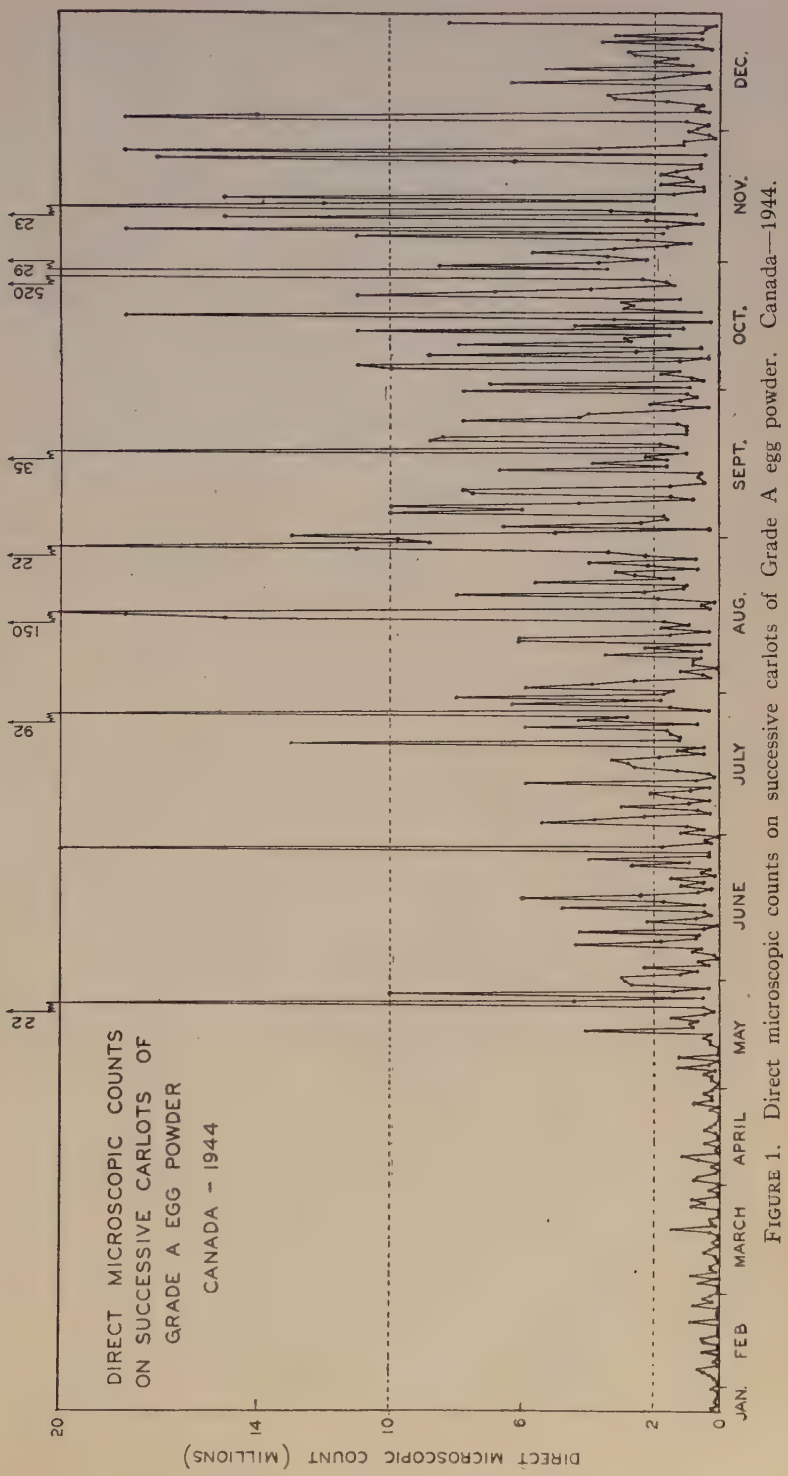

FIGURE 1. Direct microscopic counts on successive carlots of Grade A egg powder. Canada—1944.

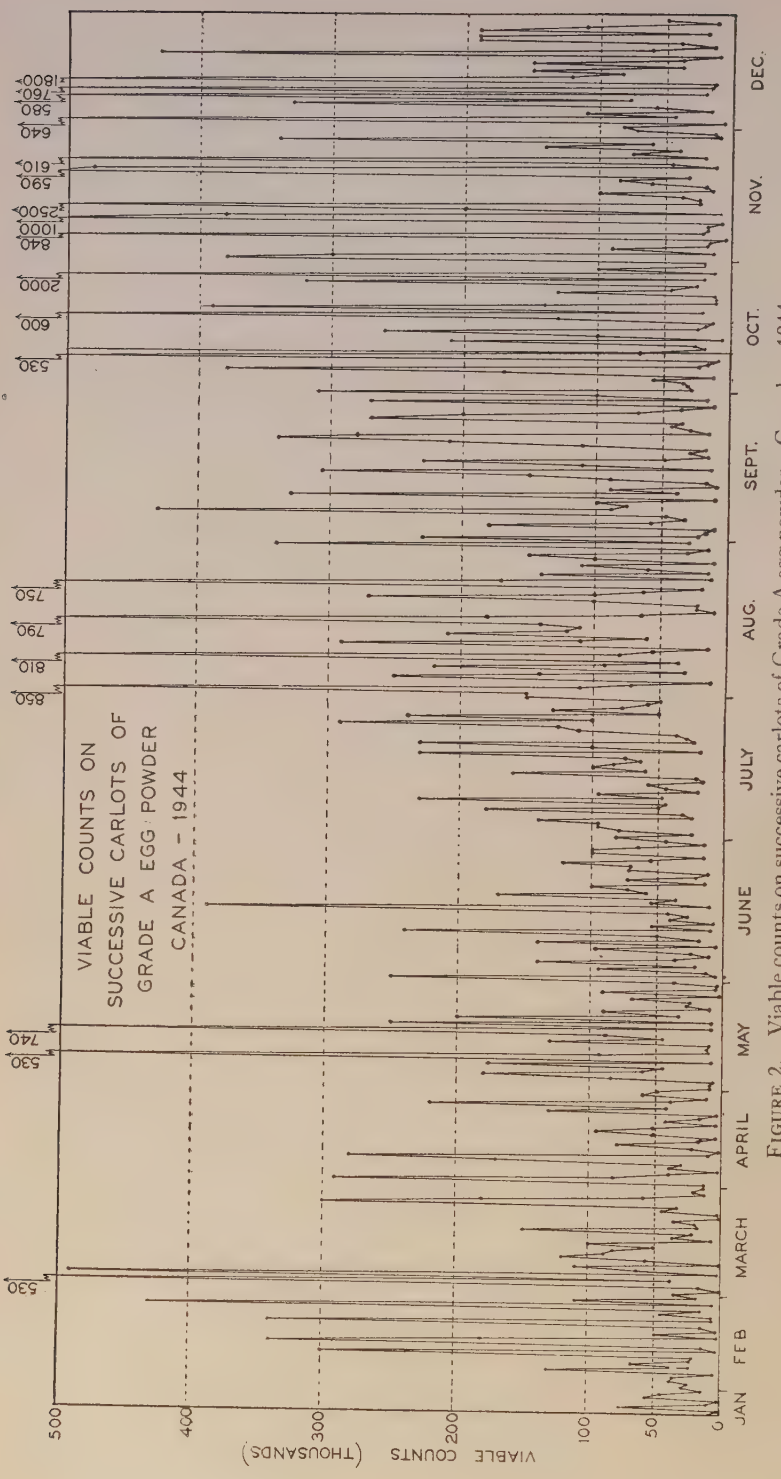

FIGURE 2. Viable counts on successive carlots of Grade A egg powder, Canada—1944.

16 hours before being dried. With the slow rate of cooling of such large masses of viscous material in still air (2), considerable bacterial development could be expected.

This plant had two cars of eggs in storage; both had been "spotted" on May 15th and were held "on track" until unloaded on the 22nd. At that time temperatures of 60° and 65° F. were recorded inside the cars. These eggs were then placed in storage at 30° F. In order to get some idea of the bacterial content, it was decided to select 5 representative cases from each carlot, and to have these eggs broken by experienced breakers using specially sterilized equipment. This was done on May 29th. Six pailfuls of melange were then selected from each carlot, mixed as thoroughly as possible by beating with long handled spoons, and samples taken for analysis. From each sample a Burri slant was prepared and incubated at room temperature for three days. In addition, a direct microscopic smear was made using the technique of Mallmann and Churchill (12).

Because of the uneven distribution of bacteria on these smears, it was not possible to draw valid conclusions concerning the bacterial contents by microscopic examination. The Burri slants, however, (Table 4) indicated marked variations from one pail of melange to the next. Furthermore, it was significant that the high count slants showed practically pure cultures, in striking contrast to the heterogeneous flora obtained from swab tests on on breaking equipment (Figure 3). This strongly suggested that the high counts were attributable to the inclusion of an occasional egg which, while normal in odour and appearance, contained enormous numbers of bacteria.

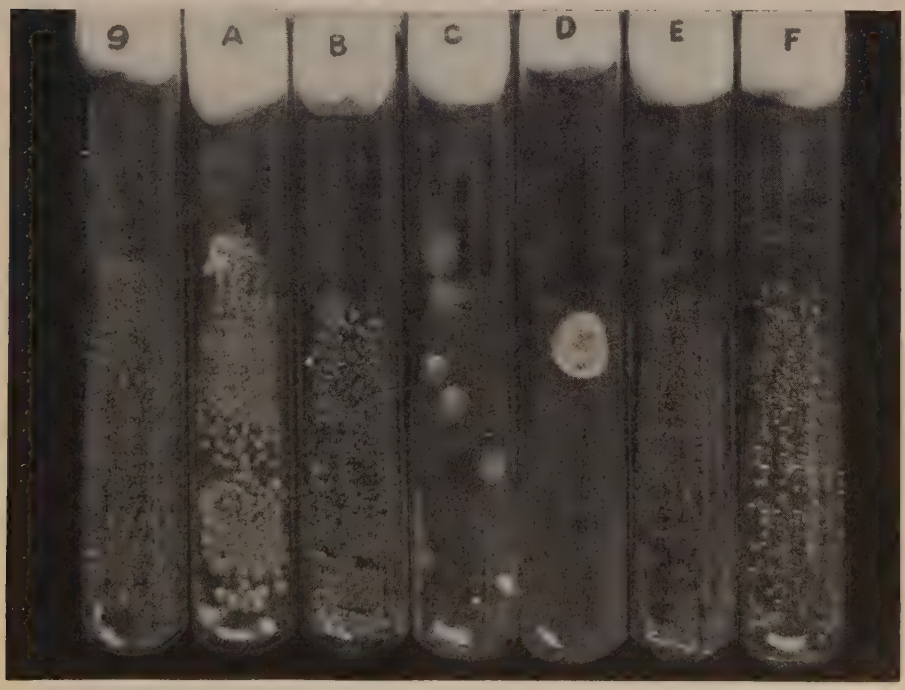

FIGURE 3. Burri slants prepared from pails of melange (A to F) and from swabbing of a breaking cup (9).

Evidence pointing in this direction accumulated as the summer went on. Burri slant determinations made at breaking plants throughout Canada showed a much higher level of counts from May on without any evidence of a let-down in sanitation in the plants. Again, the chemist at one Ontario drying plant frequently broke out 90 to 100 eggs from a current carlot. Each egg was carefully checked for abnormality, then a Burri slant prepared. Occasionally the slant from a single apparently normal egg would be so heavily overgrown that it could not be counted, while the remainder would show few, if any, colonies. Slants made after beating the entire batch of eggs in a sterilized pail would show counts in the millions, and of the type of organism found on the slant from the high count egg. At this plant, later on in August, the writer prepared triplicate Burri slants from 30 pails of melange broken out with special precautions to reduce contamination, with the results shown in Table 5. Here again it was found that the high count slants showed practically pure cultures, strongly suggesting that a single egg was responsible for the high count on the pailful of melange.

Although a number of independent breaking plants broke and froze eggs for subsequent drying, the volume of eggs handled by the Special Products Board in 1944 was so great[4] that from early May on it was impossible to find sufficient first-class shell egg storage. Unfortunately, this coincided with the appearance of unusually warm weather, temperatures approaching or exceeding 90° F. being recorded in May from Quebec to Alberta. As a result, eggs were held for longer periods at higher temperatures than are recommended. Under these conditions, it is understandable that organisms capable of infecting and growing in the egg might multiply enormously before the egg was placed in proper cold storage. Subsequent growth would depend upon the ability of the invading organism to grow at around 30° F. The presence of a small proportion of such eggs, which could not be detected by appearance or odour, would account for the marked rise in counts noted.

EXPERIMENTS WITH ORGANISMS ISOLATED FROM HIGH COUNT EGGS OR MELANGE

In order to determine the infective ability of organisms isolated from individual eggs or carefully prepared melange, fresh eggs were obtained on several occasions through the courtesy of the Poultry Division, Central Experimental Farm. These were warmed for several hours at body temperature, then immersed for 1 to 2 minutes in cold suspensions of the organisms to be tested, after the technique devised by Haines and Moran (4) for infecting eggs without rupturing the shell. The cultures employed included species of *Achromobacter*, *Flavobacterium* and *Pseudomonas*. In the earlier experiments the eggs, after immersion in the bacterial suspensions, were held at 58 to 60° F. with a relative humidity of 50%. From 4 to 5 weeks later the eggs were broken out individually, using an ordinary egg breaking knife and cup which were washed, then treated in Roccal solution (1 : 500) for 2 minutes, after each using. The broken-out eggs were placed in sterile jars and examined for odour and appearance by members of the laboratory staff as well as by specialists from the Poultry Products Division,

[4] Purchases during the first 6 months of 1944 were 212% of those for the same period of 1943.

Marketing Service, Department of Agriculture. On one occasion, 2 eggs infected with an *Achromobacter* species, and showing plate counts after 3 days at 86° F. (30° C.) of 2,500,000,000 and 2,800,000,000 per gram, respectively, had a definitely "off" odour, together with weak or stuck yolk. All other eggs were passed as being of acceptable quality, yet counts as high as 1,400,000,000 per gram were obtained (Table 6).

In a later series of tests, fresh eggs were exposed to infection as previously described, held for 1 week at 58° F., then at 40° F. for 7 weeks before being broken out. In this experiment, only the eggs exposed to the *Pseudomonas* cultures were found to contain significant numbers of bacteria (Table 7). Subsequent tests on the growth range of the various cultures used in inoculation experiments showed only the *Pseudomonas* species to be capable of strong growth at 40° F. or lower. The presence of the *Achromobacter* and *Flavobacterium* species in very large numbers in apparently normal eggs (Table 6) is strong presumptive evidence that the eggs in question had been stored at temperatures well above 40° F. for considerable periods before going into proper shell egg storage.

DETECTION OF HIGH COUNT "NORMAL" EGGS BY THEIR FLUORESCENCE

While it is now generally accepted that the majority of eggs as laid are free from micro-organisms, the possibility that eggs may carry large numbers of bacteria without any evident change in odour or appearance has been recognized (5, 14). In the Southwestern section of the United States it is not uncommon during wet weather in early spring to find such eggs in significant numbers (13). Since the majority of the bacteria isolated from such eggs are species of *Pseudomonas*, and since the members of this genus are known to produce substances fluorescing under ultra-violet light, the possibility of detecting such eggs at the time of breaking has been explored by some of the larger egg-breaking concerns there. It has been reported (13) that in one plant where special ultra-violet lamps replaced the ordinary illumination in the breaking room, it was possible, by rejection of eggs showing fluorescence, to reduce the counts on melange to one-fortieth of the previous count. While the majority of the cultures isolated in our laboratory were not *Pseudomonas* species, it seemed worth while determining the value of the ultra-violet lamp in the detection of these apparently normal high count eggs in Canada.

Through the courtesy of Swift and Company, Chicago, one of the special ultra-violet lamps from their Research Laboratories was made available for tests on storage eggs at Plant H. An assistant, whose knowledge of egg quality was well above average, was assigned to break out the eggs. As wide a selection as possible was obtained by picking a few eggs from each case as they were transferred to the shell egg buckets. Each egg was broken separately into a special black enamelled cup and checked for odour, appearance and fluorescence. If normal in odour and appearance, yet showing fluorescence, the degree of fluorescence was estimated and the egg transferred to a sterile screw-capped jar. It was then shaken vigorously to emulsify it, and a Burri slant prepared. The knife and cup were replaced each time a fluorescent egg or "reject" was encountered. In order to check on the possibility of high counts from non-fluorescent eggs, 69 eggs showing no fluorescence and 19 showing doubtful fluorescence were

examined bacteriologically. Burri slants were incubated at 70° to 80° F. (21° to 27° C.) for 3 days before being counted.

The results obtained from the examination of 240 eggs are summarized in Table 8. Although there is some correlation between count and degree of fluorescence, there are many discrepancies. If an egg showing 3 + or greater is regarded as definitely fluorescent, 31.3% of such eggs had counts under 2,000, 53% under 10,000, and 75% under 40,000 per gram. Thus if all eggs showing definite fluorescence were discarded it would mean the rejection of a considerable percentage which were otherwise acceptable.

On the other hand, the data show that high count eggs do not always show fluorescence. Results obtained in the analysis of 348 storage eggs in August, 1943 (6) showed only 2.3% with counts in excess of 40,000 per gram. Taking this as the maximum acceptable count, it will be seen that in the present studies only 8 of the 28 eggs in this group showed definite fluorescence as defined above. Among the remaining 20 eggs are two with counts of more than 10,000,000 and 20,000,000 per gram, respectively. While the elimination of those eggs showing strong fluorescence would help in reducing the count, it would not prevent the occasional acceptance of eggs with very high counts, while at the same time rejecting a number of acceptable eggs.

In our studies with cultures isolated from apparently normal eggs, definite fluorescence was noted on eggs experimentally infected with *Achromobacter* and *Flavobacterium* species as well as with *Pseudomonas* when held at 58 to 60° F. (Table 6). To determine whether there was any correlation between the type of organism and the degree of fluorescence of the naturally infected egg (Table 8), cultures isolated from high count eggs were identified as to genus. The results (Table 9) indicate no definite correlation. *Achromobacter* and *Flavobacterium* species were isolated from eggs showing medium to strong fluorescence, while 1 of the 3 eggs from which a *Pseudomonas* species was isolated showed little or no fluorescence. However, the bacterial contents of the eggs infected with *Pseudomonas* species were much lower than those generally encountered where *Achromobacter* or *Flavobacterium* species were found. It is of interest to record that although the *Pseudomonas* species were the only ones showing strong growth at 29° F. (−2° C.) within 2 weeks, all but one of the other cultures showed moderate growth at this temperature after 5 weeks, while all showed good growth at 40° F. (4° C.) after 1 week.

THE PRESENCE OF STREPTOCOCCUS FAECALIS IN EGG POWDER

In view of the assertion that *Streptococcus faecalis*, an organism commonly isolated from the intestinal contents of man and other animals, is one of the commonest species found in egg powder, two representative colonies were picked from plates poured from 38 Grade A and 12 Grade B carlot samples. These included powder from each of 8 drying plants. Of 96 such cultures studied in detail, only 3 proved to be *S. faecalis*. As had been found in 1943, the commonest type of organism appearing on plates of tryptone-glucose-extract-skimmilk agar incubated for 48 hours at 37° C. (98.6° F.) was a streptococcus, so far unidentified, which forms small amounts of acid but fails to curdle litmus milk at room temperature or 30° C. (86° F.).

DISCUSSION

The experience of the past summer suggests the need for revising our opinion regarding the sanitary significance of high direct microscopic counts in dried whole egg powder. Previously we would have been in entire agreement with the statement of Lepper, Bartram and Hillig (11) that, "In no instance did dried eggs show a microscopic count exceeding 10 million per gram . . . . . when they were prepared from sound raw material. In all cases where these counts were exceeded, decomposed or rotten eggs had been incorporated in the product or the eggs had been subjected to conditions after breaking-out which permitted them to sour." For at least some of the high count samples examined here in 1944, we have reason to believe that this statement would not hold true. There was no temptation for a dryer to use sub-standard eggs, since the Special Products Board furnished graded eggs and maintained a resident inspector at the plant. Furthermore, the dryer was faced with a stiff financial penalty if his product failed to meet the bacteriological specifications. Consequently, dryers were very particular about the quality of eggs broken out.

Although the results obtained in 1944 indicate that some caution must be observed in interpreting high direct microscopic counts, it should not be concluded that such counts are of little value in controlling sanitation and plant practices. As will be seen from the data in Table 10, there have been several instances in which faulty practices such as inadequate cooling of melange were not reflected in the viable count or pH value of the powder, but were detected through the direct microscopic count. High microscopic counts due to the eggs themselves are largely, if not entirely, made up of rod forms; those due to faulty plant practices, on the other hand, are usually made up mainly of paired cocci, resembling the picture obtained in souring milk. This distinction has often proven to be of real value when the cause of a high count is being sought.

The direct microscopic count is particularly valuable in that it does not appear to be affected by conditions of drying and subsequent storage to the same degree as is the viable count. Plants vary greatly in the degree of bacterial destruction brought about by the drying process, as indicated by the data shown in Table 11. Without the direct microscopic count, some plants would be credited with doing a much better job than they are actually doing, while the converse would hold true for others. Studies intended to throw some light on the reasons for these differences in bacterial destruction between plants are under way during the 1945 drying season.

SUMMARY

As judged by plate counts, the bacteriological condition of Canadian dried eggs in 1944 was very satisfactory.

Unusually high direct microscopic counts were noted from May on. While a few of these were attributable to inadequate cooling of melange due to refrigeration failures, the majority were due to the inclusion of a small percentage of eggs which, while apparently normal in appearance and odour, yet contained enormous numbers of bacteria. Fluorescence under ultra-violet light was of limited value in the detection of such eggs.

While a high direct microscopic count cannot always be regarded as an indication of faulty plant practice, the method can yield information in this regard which is not always obtainable through the plate count or pH value.

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Table 1.—Monthly arithmetical averages of bacteria counts for Canadian grade A dried whole egg powder, 1943-1944

Month	Plate count (thousands per gram)		Direct microscopic count (thousands per gram)	Percentage over 2,000,000
	1943	1944	1944	
January	84	27.5	130	0
February	409	82	250	0
March	160	86	321	0
April	120	58.4	267	0
May	80	75.5	1,785	17.1
June	139	68	1,652	25.5
July	90	115	4,642	37.2
August	130	112	3,522	48.9
September	94	119	3,998	47.7
October	102	101	4,436	62.5
November	681	143	4,595	42.5
December	376	131	1,810	33.3

TABLE 2.—BACTERIA COUNTS ON CONSECUTIVE CARLOT SAMPLES OF GRADE A POWDER FROM WESTERN CANADIAN PLANTS

Plant	Date analysed	Count per gram	
		Direct microscopic	Plate
A H–18	April 22	< 70,000	17,000
J– 2	May 3	< 70,000	9,800
J–12	May 13	< 70,000	12,000
J–19	May 24	22,000,000 Y	28,000
J–30	June 3	340,000	22,000
B H–10	April 15	70,000	18,000
H–24	May 1	150,000	9,500
J–12	May 13	290,000	10,000
J–24	May 29	2,700,000	4,900
K– 9	June 15	2,200,000	7,100
K–17	June 17	6 000,000 Y	36,000
K–23	June 23	590,000	12,000
C H–30	May 4	220,000	83,000
J– 5	May 8	1,300,000	44,000
J–10	May 15	510,000	45,000
J–18	May 25	4,400,000 Y	25,000
J–25	May 23	440,000	33,000
J–30	June 2	660,000	13,000
K– 5	June 7	70,000	37,000
D J– 1	May 3	220,000	6,800
J– 4	May 9	< 70,000	8,100
J–10	May 12	< 70,000	11,000
J–14	May 16	810,000	7,900
J–17	May 19	660,000	8,700
J–22	May 24	510,000	10,000
J–23	May 27	10,000,000 Y	2,300
J–26	May 27	340,000	5,300
E H–20	April 26	810,000	220,000
J– 4	May 9	1,300,000	530,000
J–15	May 16	4,100,000	740,000
J–27	May 31	2,700,000	37,000
K– 6	June 7	810,000	25,000
K–20	June 29	220,000	65,000

Y = high count due to Y organism.

TABLE 3.—BACTERIA COUNTS ON SPECIAL SAMPLES OF GRADE A POWDER FROM TWO WESTERN CANADIAN PLANTS, 1944

Plant	Time of sampling	Direct microscopic count	Plate count
A	Start of run, May 25	19,000,000 Y	8,600
	Finish of run, May 25	23,000,000 Y	23,000
	Start of run, May 26	18,000,000 Y	9,100
	Finish of run, May 26	140,000,000 Y	83,000
	Start of run, May 27	17,000 000 Y	10,000
	Finish of run, May 27	7,300,000 Y	88,000
C	Start of run, May 26	17,000 000 Y	8,300
	Finish of run, May 26	6,600,000 Y	38,000
	Start of run, May 27	21,000,000 Y	20,000
	Finish of run, May 27	8,400,000 Y	20,000
	Start of run, May 28	7,000,000 Y	10,000
	Finish of run, May 28	12,000,000 Y	11,000

Y = high count due to Y organism.
Average length of run = 21 hours.

TABLE 4.—COUNTS OBTAINED BY BURRI SLANT METHOD ON PAILS OF MELANGE BROKEN OUT WITH SPECIAL PRECAUTIONS. PLANT E, MAY 29, 1944

Lot No.	Pail No.	Count per gram
2529	1	1,400,000
	2	< 2,000
	3	4,000
	4	10,000
	5	20,000
	6	320,000
2536	1	300,000
	2	22,000
	3	58,000
	4	10,000
	5	600,000
	6	4,000

TABLE 5.—COUNTS OBTAINED BY BURRI SLANT METHOD ON PAILS OF MELANGE BROKEN OUT WITH SPECIAL PRECAUTIONS. PLANT H, AUGUST 23-24, 1944

Lot No.	2172	3204	2167	2167	2167
Time of sampling	23rd a.m.	23rd p.m.	24th a.m.	24th a.m.	24th a.m.
Pail No.	Count per gram				
1	2,000	3,700,000	800,000	8,000	2,000
2	1,000	3,200,000	1,100,000	2,400,000	3,800,000
3	1,600,000	1,400,000	7,000	900,000	1,600,000
4	1,500,000	4,000,000	2,000,000	3,000,000	4,000
5	1,000	1,100,000	4,000,000	3,800,000	1,200,000
6	1,300,000	1,200,000	16,000	700,000	46,000

TABLE 6.—RESULTS OF EXAMINATION OF EGGS EXPOSED TO INFECTION WITH CULTURES OF BACTERIA ISOLATED FROM APPARENTLY NORMAL EGGS

Culture	Species	Storage conditions	Fluorescence	Plate count per gram
A	*Achromobacter*	4 weeks, 58-60° F.	+++	2,500,000,000
B 1	*Flavobacterium*	4 weeks, 58-60° F.	?	300,000,000
E	*Pseudomonas*	4 weeks, 58-60° F.	?	120,000
K	*Pseudomonas*	4 weeks, 58-60° F.	++++	260,000,000
N	*Achromobacter*	4 weeks, 58-60° F.	?	160,000,000
A	*Achromobacter*	5 weeks, 58-60° F.	?	2,800,000,000
B 1	*Flavobacterium*	5 weeks, 58-60° F.	+++	86,000,000
J	*Flavobacterium*	5 weeks, 58-60° F.	?	800,000,000
K 5	*Flavobacterium*	5 weeks, 58-60° F.	?	1,400,000,000
R	*Achromobacter*	5 weeks, 58-60° F.	++	84,000,000

TABLE 7.—RESULTS OF EXAMINATION OF EGGS EXPOSED TO INFECTION WITH CULTURES OF BACTERIA ISOLATED FROM APPARENTLY NORMAL EGGS*

Culture	Species	Appearance and odour	Fluorescence	Bacteria per gram
B 1 a	*Flavobacterium*	O.K.	—	8,000
b	*Flavobacterium*	O.K.	—	< 2,000
E a	*Pseudomonas*	White sl. green	+++++	720,000,000
b	*Pseudomonas*	O.K.	—	< 2,000
G a	*Pseudomonas*	White sl. green	++++	310,000,000
b	*Pseudomonas*	White sl. green	++++	430,000
J a	*Achromobacter*	O.K.	—	< 2,000
b	*Achromobacter*	O.K.	—	< 2,000
K a	*Pseudomonas*	White sl. green	++++	480,000,000
b	*Pseudomonas*	White sl. green	++++	120,000
N a	*Achromobacter*	O.K.	—	< 2,000
b	*Achromobacter*	O.K.	—	< 2,000
R a	*Achromobacter*	O.K.	—	< 2,000
b	*Achromobacter*	O.K.	—	< 2,000
Control a	—	O.K.	—	< 2,000
b	—	O.K.	—	< 2,000

* Eggs held for 1 week at 58° F., then for 7 weeks at 40° F. before being broken out.

TABLE 8.—CORRELATION BETWEEN FLUORESCENCE AND BACTERIAL CONTENT OF STORAGE EGGS BROKEN OUT NOVEMBER 24, 1944

Bacteria count per gram	No. of eggs	Degree of fluorescence						
		−	+?	+	++	+++	++++	+++++
< 2,000	120	34	8	45	23	9	1	
2,000 – 10,000	69	22	4	19	17	7		
11,000 – 50,000	25	8	3	1	5	7		1
51,000 – 200,000	14	6	3	2	3			
201,000 – 1,000,000	5		1		2		2	
1,010,000 – 5,000,000	1						1	
>5,000,000	6			1	1	2	2	
	240	70	19	68	51	25	6	1

TABLE 9.—RELATIONSHIP BETWEEN BACTERIA COUNT AND FLUORESCENCE OF APPARENTLY NORMAL EGGS AND THE CHARACTERISTICS OF THE ORGANISMS ISOLATED THEREFROM

Culture No.	Genus	Growth −2° C. in 2 weeks	Bacteria count per gram	Degree of fluorescence of	
				Egg*	Culture†
T 1	*Achromobacter*	—	>20,000,000	++	—
T 2	*Achromobacter*	—	>10,000,000	+	—
T 3	*Achromobacter*	—	280,000	?	—
T 12	*Achromobacter*	—	>10,000,000	+++++	—
T 18	*Achromobacter*	—	9,000,000	+++++	—
T 6	*Flavobacterium*	+	8,000,000	+++	—
T 123	*Flavobacterium*	—	10,000 000	+++	—
T 10	*Pseudomonas*	+++	450,000	+++++	+++
T 97	*Pseudomonas*	+++	66,000	?	+++
T 116	*Pseudomonas*	+++	240,000	+++++	+++

* Determined by U.-V. light at the moment of breaking.

† Determined by U.-V. light on 24-hour growth in Georgia & Poe's asparagine medium.

TABLE 10.—DATA ON SAMPLES WHERE HIGH COUNTS WERE BELIEVED TO BE DUE TO FAULTY PLANT PRACTICES

Plant	Carlot No.	pH Value	Plate count	Direct microscopic count
B	L–20	8.68	100,000	13,000,000
	L–25	8.65	240,000	92,000,000
C	K–26	8.70	100,000	20,000,000
	M–30	8.72	230,000	13,000,000
	O–25	8.54	2,000,000	520,000,000
F	M–17	7.82	64,000	150,000,000
	N–14	8.57	29,000	35,000,000
	P–24	8.58	39,000	18,000,000
G	M–14	8.67	790,000	15,000,000
	O–14	8.54	260,000	11,000,000
	O–20	8.61	500,000	18,000,000

TABLE 11.—EFFICIENCY OF BACTERIAL DESTRUCTION AS JUDGED BY RATIOS OF PLATE COUNTS TO DIRECT MICROSCOPIC COUNTS ON DRIED WHOLE EGGS

(October-December, 1944)

Plant	Carlot No.	Plate count per gram	Direct microscopic count per gram	Ratio
E	O– 9	390,000	2,900,000	1 : 7.4
	O–23	26,000	1,400,000	1 : 53.8
	O–31	380,000	2,200,000	1 : 5.8
	P– 9	1,000,000	2,000,000	1 : 2.0
	P–17	590,000	1,800,000	1 : 3.1
	P–25	340,000	1,100,000	1 : 3.2
	Q– 5	330,000	510,000	1 : 1.5
	Q–14	150,000	810,000	1 : 5.4
	Q–21	110,000	440,000	1 : 4.0
			Average	1 : 9.6
F	O– 9	27,000	7,900,000	1 : 292.6
	O–22	12,000	11,000,000	1 : 916.6
	O–30	13,000	5,700,000	1 : 438.5
	P– 9	24,000	12,000,000	1 : 500.0
	P–18	20,000	17,000,000	1 : 850.0
	P–24	39,000	18,000,000	1 : 461.5
	Q– 5	20,000	3,400,000	1 : 170.0
	Q–18	18,000	3,200,000	1 : 177.7
	Q–26	50,000	8,200,000	1 : 164.0
			Average	1 : 441.2

THE DIAGNOSIS OF SEX BY MEANS OF HETEROPYCNOSIS[1]

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The Basic Problem

In countries that have taken the appropriate statistics it is found that a gradual and progressive change occurs in the secondary sex ratio of man running from 104–107 ♂♂ : 100 ♀♀ at birth to a preponderance of females that approaches 2 : 1 at extreme senescence. Furthermore, by taking these post-uterine data together with the sex ratio obtaining among still births, premature births and abortuses, evidence is adduced by extrapolation pointing to a primary sex ratio, or that obtaining at conception, approaching 150 : 100. Expressed very roughly, then, the sex ratio changes between conception and extreme old age from 150 : 100 to 50 : 100. The conclusion that the change is due to a constitutional weakness of the male relative to the female is supported by a consideration of the influence of various environmental conditions (Crew, 4).

These facts pose two distinct problems: *first, what factors are concerned in the observed sexually selective mortality that operates against the male? second, what is the underlying cause of the initial inequality of the two types of zygotes?*

Hypotheses Regarding Differential Mortality

Three obvious factors have been suggested as contributing to differential mortality: (1) the operation of sex-linked genes that are disadvantageous, deleterious or lethal; (2) sex-limited defects and derangements; and (3) differences in the relative metabolic rates of males and females. Now since a similar preponderance of male live births holds for most domestic mammals, and since in all mammals it is the male that is the heterogametic sex, it is impossible, on the evidence presented by them, to decide whether the inherent weakness of the male results purely and simply from his maleness or whether it results from his heterogametic constitution.

Hypotheses Regarding Inequality of Sexes

Regarding the second problem, the numerical inequality of the two sexes at conception, various solutions have been advanced. Clearly, on the strength of the extrapolation mentioned above, the Y-containing sperm is successful in fertilization to an extent approaching, in man, about one and one-half times that of the X-containing sperm. One suggestion regarding this is that the Y-sperm is produced proportionately more often, but this appears unlikely from observations of meiosis in the human male. However, instances of excess X-sperm production are known in Drosophila (Morgan, Bridges and Sturtevant, 14) in which the male is likewise heterogametic. Another, no more attractive, involves selective fertilization controlled by the female. Yet another suggests that the Y-sperm should function more readily since its mass should be less than that of the X-sperm.

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This theory, put forward by Morgan (13), has been more favourably received because of the claim (Parkes, 15) that a bimodal curve results from plotting the head size of human sperm. To these may be added the possibility that, in view of the greater amount of active chromatin carried by the X relative to the Y-chromosome and its consequently greater mutation potential, the X-sperm may be more subject to the occurrence of mutations deleterious to the functioning of the gamete. Other things being equal, the X-sperm would be under a temporal handicap in the race to accomplish fertilization.

The Natural Experiment

In birds, certain fishes, and butterflies and moths, unlike all other known animal organisms (with the possible exception of the Trichoptera (Klingstedt, 10)), it is the female that is heterogametic. With this cytogenetic peculiarity firmly established, it soon became evident that they constitute material for a natural experiment by which to evaluate some of the possible solutions to the above two main problems. In the first place it becomes possible to determine the parts played by genetic and other factors when mortality is differential. If it is the heterogametic sex, regardless of whether this is male or female, that dies off more readily, the cause must rest in its genetic constitution; if, on the other hand, it is the male sex that is less viable, regardless of whether it is heterogametic or homogametic, maleness, as such, must be held responsible. In the second place, since with homogametic males there can be no choice between sperm, any numerical inequality of the two sexes at conception must be the direct responsibility of the female; one type of egg must be produced or must function in excess of the other. Both the possibility of different rates in the movement of sperm and that of differential selectivity by the female, as advocated in the case of humans, are here immediately ruled out for the simple reason that only one type of sperm is formed. To test for and between differential production and differential functioning of ova, it becomes necessary to determine whether, in heterogametic females, the primary sex ratio is other than equality.

Practical considerations usually limit the diagnosis of sex to those stages in the life cycle after the formation of the definitive gonad (but see later). Only in the complete absence of mortality during the earlier stages can this value be taken as the primary sex ratio.

In the attempt to arrive at both the true primary sex ratio and the initial gametic proportions, birds, fishes and lepidopterans all have their own particular disadvantages. Birds normally have a relatively low reproductive rate; the cytological constitution of most fishes is unknown; and Lepidoptera in general suffer from extreme mortality before sex can be easily determined. Of the three, however, the Lepidotera are by far the most easily manageable and readily offer themselves in numbers upon which statistical reliance can be placed. Furthermore, in their diversity of egg-laying habits, they allow, by the selection of technically suitable species, the determination, not only of percentage mortality, but also the sequence in which viable and non-viable eggs are laid. Finally, as a result of the mechanics of egg-production in insects, the possibility of differential functioning can be tested and, if found inoperative, the degree of differential

production can be evaluated. The chief limitation, until now, has been the gap created by the delay in sex-differentiation; the greater the gap, the greater the part mortality can play, if differential, in distorting the sex ratio. As will be shown, we now have a method of narrowing the gap and, by the appropriate selection and classification of material, of closing it completely.

The Method of Approach

It is a well-known characteristic of sex chromosomes that in the heterogametic sex they often retain their staining capacity throughout the division cycle, while the autosomes, along with the sex chromosomes in the homogametic sex, are non-stainable during the resting stage. It should therefore be possible to use this phenomenon of heteropycnosis in the diagnosis of sex at stages prior to that at which anatomical differences are developed. Other cytological criteria are theoretically available but, if the sex chromosomes should display heteropycnosis in somatic cells, attention can then be confined to the resting nuclei, leaving the observer independent of numerical or size differences and angle of vision.

Application of Method and Results

The first step was to test for somatic heteropycnosis in lepidopterous larvae in which sex could be predetermined by external examination. The species selected for this purpose was *Archips fumiferana*, the spruce budworm, which for the past few years has again been occurring in extreme outbreak proportions over a large area of northeastern North America. In this species the sex can be determined by simple observation as the gonads of the male are clearly visible through the integument of the fourth and later instars. Temporary aceto-carmine slides and permanent Feulgen "squash" preparations (Smith, 18) made from the intestine showed deeply

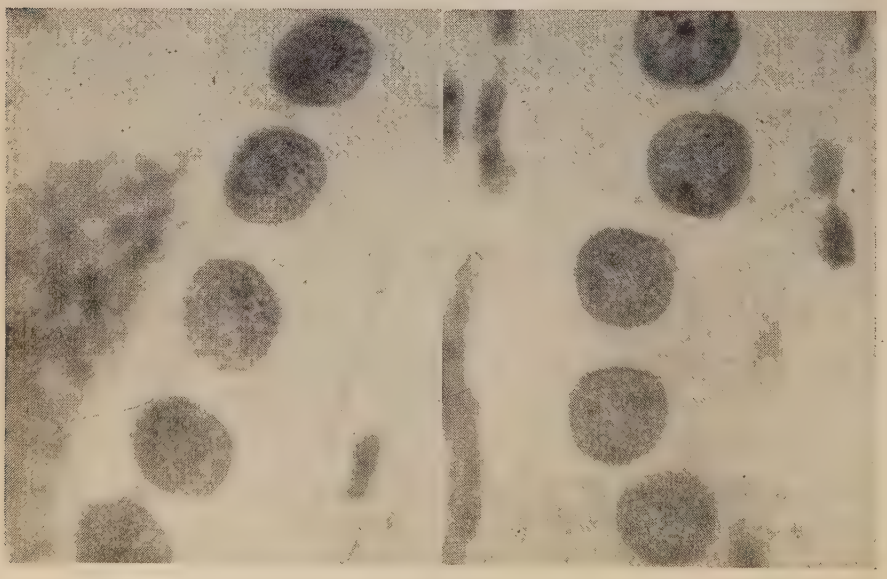

FIGURE 1.

staining bodies in the resting nuclei of each of 17 female larvae studied, while they were absent from all the 12 males examined. These heteropycnotic bodies, restricted to the resting nuclei of females, are obviously the the sex chromosomes and may therefore be used in the diagnosis of sex.

The method, slightly modified, was then applied to 399 freshly eclosed progeny of eight budworm pairs collected *in copula* from the wild. The modification facilitated the handling of the small larvae in the large numbers being dealt with. It consisted in fixing, embedding and sectioning families en masse rather than making individual preparations[3]. Also it was found more convenient to examine the relatively enormous cells of the silk glands (see Figure 1) since their use did away with the necessity of using the oil immersion objective. The results are tabulated below:

Family number	Total eggs laid	Total eggs unhatched	Percentage mortality	Heteropycnosis		Sex ratio
				Without	With	
						♂ ♀
8	53	0	0	25	28	89 : 100
7	64	2	3	28	34	82 : 100
10	90	8	9	35	47	74 : 100
6	49	8	16	18	19	95 : 100
13	98	19	19	35	37	95 : 100
1	34	9	26	5	20	25 : 100
21	67	25	37	22	16	137 : 100
14	83	53	64	5	25	20 : 100
	538	124	23	173*	226*	76 : 100
			Expected	199.5	199.5	

$X^2 = 7.04$, P = *ca* .01

* The discrepancy between the number of larvae hatched and the number scored for heteropycnosis is due solely to the fact that the last 15 larvae to hatch were not embedded due to pressure of work.

[3] I am indebted to Dr. W. O. Rothwell, Temiskaming Hospital, for providing the facilities for embedding the material described herein.

There is no intention of analysing these results to determine whether the primary sex ratio in Lepidoptera, as represented by *Archips fumiferana*, is other than equality. They have been presented, to show, first, that the phenomenon of somatic heteropycnosis, when the property of a species, can be used in the early diagnosis of sex and, secondly, that direct evidence can be obtained regarding the primary sex ratio and the effect on it of differential mortality. Of course, the present results require considerable amplification.

Limitations of Method

To what extent the method can be applied to other organisms is at present unknown for, although much work has been done on heteropycnosis since it was first noted by Henking (7) in 1891, remarkably few generalizations can be made concerning it. Thus almost 40 years after Henking's original observations Schrader (16, p. 8) had perforce to quality the statement that "It is of interest to observe that this special behaviour of the sex chromosomes is not manifested prior to the spermatogonial stages," as

follows—"However, it must not be forgotten that researches touching on the sex chromosomes in somatic cells are small in number and generalization is not justified."

There are certainly many circumstances that curtail the general use of the method. For example, in the only extensive survey published to date, Geitler (6) found, in the Heteroptera, that somatic heteropycnosis is not the exclusive property of the sex chromosomes of one sex in all species. This is in agreement with Kaufmann's (8) observations on *Drosophila melanogaster* where the sex chromosomes in the somatic cells of both the male and the female exhibit heteropycnosis, and is supported by the writer's (unpub.) survey of Coleoptera in which both sexes of certain species are found to be devoid of this property. In the Acrididae the sex chromosomes fail to display heteropycnosis until the spermatogonial divisions (Mohr, 11, 12) but later, during the meiotic prophase, certain autosomes or parts of autosomes (Carothers, 2) may show a similar but less intense reaction. Again heteropycnosis is normally the property of odd supernumerary chromosomes at meiosis (Wilson, 19) but fails to express itself when the supernumerary is present in duplicate (Carroll, 3). Conditions in the Lepidoptera are variable. Thus both Seiler (17) and Dederer (5), working on *Phragmatobia* and *Philosamia*, respectively, report that during meiosis the sex chromosomes of the female are indistinguishable from the autosomes. Kawaguchi (9), however, finds that they are heteropycnotic during the meiotic prophase in some species but not in others. In *Archips fumiferana* no indication of heteropycnosis has been found during either oogenesis or spermatogenesis (unpub. data) even though its occurrence in somatic tissues of the female is beyond dispute.

Advantages of Method

In conclusion, it might be pointed out that, although there appear to be technical limitations to the use of the method due to the apparent need for specialized equipment, it is possible to dispense with sectioning by using aceto-carmine staining. However, the practice of mass embedding and sectioning is less time-consuming and less tedious. The alternative method of diagnosing sex by dissecting for primordial gonads is unfortunately, even where possible, likely to leave a considerable unclassifiable residue, whereas using heteropycnosis there is none. Thus Brandt (1) found it impossible to classify more than 537 out of 717 first instars of *Lymantria monacha* on the basis of the morphology of the primordial gonads. With a surplus of only 4.6 females per hundred larvae it would obviously be worthwhile in future to attempt to sex any residual larvae by means of the aceto-carmine technique. This involves nothing more than dissecting the silk glands in a drop of aceto-carmine and examining them after a minute or so under a microscope capable of giving a magnification of only about ×300.

SUMMARY

The facts and hypotheses concerning the human sex ratio are briefly reviewed. The limitations imposed by high mortality on the use of the Lepidoptera as a "natural experiment" by which certain of the hypotheses may be crtically appraised are discussed and the possibility is demonstrated of using somatic heteropycnosis as diagnostic of sex prior to sexual differentiation and before differential mortality can distort the primary sex ratio.

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